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June 2000

CEQ™2000

DNA Analysis System

User's Guide

Genetic Analysis Development Center
4300 North Harbor Boulevard, Fullerton, CA 92835

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Foreword

About this Guide

This guide is intended for use by the user of the CEQ™2000 DNA Analysis System. It is divided into the following sections:

The full product name is CEQ 2000 DNA Analysis System. For the sake of brevity, the product name will also appear as CEQ System.

- *Foreword*, this section, describes the purpose of this guide, provides a list of its contents, discusses the use of the symbols used in the document as well as providing service contact information.
- Chapter 1, *"System Overview,"* discusses the purpose and functional description of the system, provides an overview of the three main components (chemistry, hardware and software) comprising the system and details the safety features relevant to the system.
- Chapter 2, *"Program Description,"* provides descriptions of the main windows, menus, dialog boxes and software commands controlling the system.
- Chapter 3, *"Operating the System,"* contains step-by-step procedures for all common tasks necessary to prepare and use the system on a daily basis. It also provides procedures to perform the tasks involved in analyzing/managing samples and sample data.
- Chapter 4, *"Routine Maintenance,"* provides routine maintenance procedures and biological waste disposal procedures. It also lists the consumable materials used in the system.

Symbols Used in this Guide

The following information describes the symbols used in this document. Beckman Coulter recommends that you review this information before using the CEQ 2000 System.

WARNING **When the "warning" icon accompanies text, it indicates that a potential hazard to your personal safety exists if information stated within the "Warning" paragraph is not adhered to or if procedures are executed incorrectly.**

CAUTION **Paragraphs marked by the "caution" symbol indicate that there is a potential danger of equipment damage, software program failure or that a loss of data may occur if information stated within the "Caution" paragraph is not adhered to or if procedures are executed incorrectly.**

Paragraphs marked by the "notepad" icon contain supplemental or explanatory information concerning the current topic or procedural step.

Getting Help

If you encounter a problem that is not discussed in this guide and you need technical support, contact your local dealer, the provider of this product, or contact Beckman Coulter directly using the information below.

Whenever you call your local dealer or Beckman Coulter, be sure to have your registration material, instrument serial number and software version number available. For future reference, record this information here.

Instrument Serial Number: _____

Software Version: _____

Firmware Version: _____

Software Applications: _____ **Sequence Analysis**

_____ **Fragment Analysis**

_____ **Both**

Dealer Name: _____

Dealer Phone Number: _____

Mail	Beckman Coulter, Incorporated 4300 North Harbor Boulevard Fullerton, CA 92835
Product Support	1-800-854-8067
Sales	1-800-742-2345
Service	1-800-551-1150
Telex	678413
FAX	l-800-643-4366

Internet	http://www.beckmancoulter.com
Worldwide Offices	Africa, Middle East, Eastern Europe (Switzerland) (41) 22 994 07 07 Australia (61) 2 844 6000 Austria (43) 1 72 92 164 Canada (800) 387-6799 France (33) 1 43 01 70 00 Germany (49) 89358700 Hong Kong, PRC (852) 28147431 Italy (39) 2 953921 Japan (81) 3-5352-2825 Mexico (525) 559 1635 Netherlands (31) 2972 30630 Poland (48) 22 408822, 408833 Singapore (65) 339 3633 South Africa (27) 11 805 2014/1 Spain (34) 1 358 005 Sweden (46) 8 985320 Switzerland (41) 229940707 Taiwan (886) 023783456 U.K. (44) 1 494441181 U.S.A. 1-800-742-2345

Chapter One 1

System Overview

Chapter Overview

This chapter discusses the purpose and functional description of the system, provides an overview of the three main components (chemistry, hardware and software) comprising the system and details the safety features relevant to the system.

Purpose

The purpose of the Capillary Electrophoretic (CE) Deoxyribonucleic Acid (DNA) Analysis System (CEQ) is two-fold:

1. To determine the nucleotide sequence of any given DNA sample.
2. To estimate sizes of DNA fragments.

The system is comprised of three main components: chemistry, hardware and software.

Functional Description

The CEQ 2000 DNA Analysis System is a fully automated system capable of determining the base sequence and fragment length of DNA samples that have been prepared with Beckman Coulter dye-labeled reagents. Four-color, dye-labeled terminator chemistry kits are used to process samples for base sequence analysis. Generation of samples for fragment length analysis is performed using dye-labeled primers.

The CEQ 2000 accepts up to 96 four-color samples in a microplate. Each row of eight samples (sample set), containing labeled DNA fragments, is automatically denatured and then separated by capillary electrophoresis. The replaceable medium (separation gel) is automatically replaced in the eight capillaries after each separation. The separation gel supply is an easily replaced cartridge with a capacity sufficient for a full microplate (96 samples).

Detection is by laser-induced fluorescence in four spectral channels. The four-channel raw data sets generated by each of the eight capillaries are automatically processed to produce high quality base sequences or fragment lists after separation.

Raw and analyzed data are stored in a database and may also be exported in file formats compatible with common analysis applications.

Chemistry

The chemistry (enzyme, nucleotides, and dye-labeled nucleotides) is primarily used for the preparation of the DNA sample before the electrophoretic process begins.

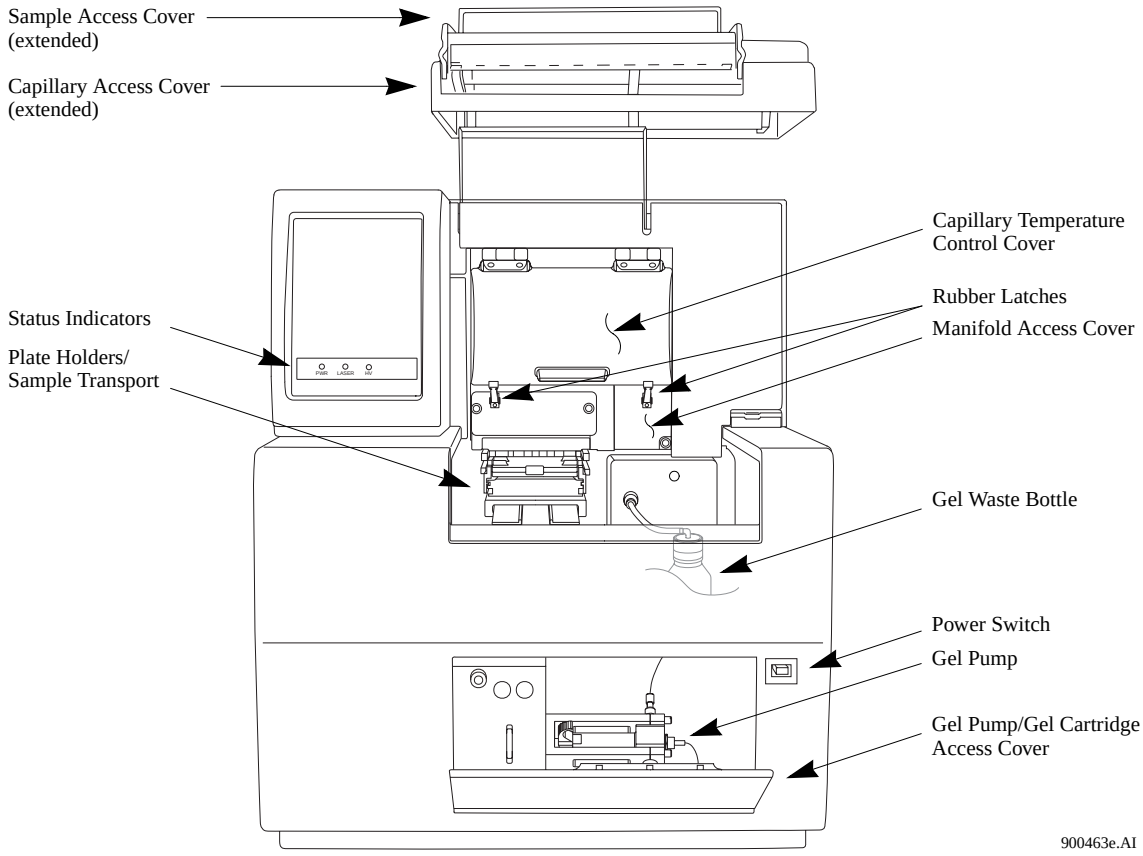
Detailed information concerning the chemistry and protocols are contained in each reaction kit.

Hardware

The hardware carries out the task of sample handling as well as tasks associated with the separation and detection phases of electrophoresis.

Figure 1 shows the CEQ instrument. The user accessible hardware components of the CEQ instrument are described below.

Figure 1: Hardware Overview



Sample Access Cover

The Sample Access Cover is a transparent cover that provides viewing and access to the Buffer Plate, Wetting Tray and Sample Plate.

Capillary Access Cover

This cover provides access to the Capillary Temperature Control Cover.

Capillary Temperature Control Cover

This cover provides an enclosed environment for the Capillary Temperature Control and allows access to the capillary array.

Capillary Array

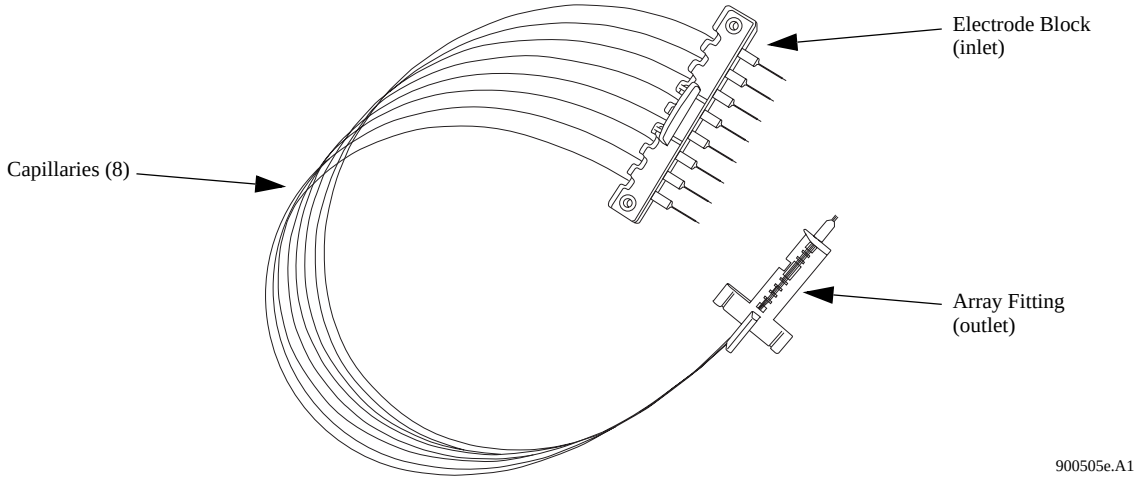
The capillary array used to produce raw data for standard sequence analysis differs from the capillary array used to produce raw data for Long Fast Read sequencing (LFR) and fragment analysis. The capillary array used for standard sequencing is 53 centimeters in length and has an internal diameter (i.d.) of 100 micrometers. The capillary array used for Fragment Analysis and Long Fast Read sequencing is 33 centimeters in length, and has an i.d. of 75 micrometers. The decrease in length reduces run time, and the decrease in i.d. decreases detection sensitivity. A different capillary heater plenum is necessary for the each capillary array. The Long Plenum Assembly is used with the 53 cm capillary array and the Short Plenum Assembly is used with the 33 cm capillary array. Therefore, the plenum must be replaced when switching from the longer to the shorter capillary array, and vice versa. A variety of capillary arrays are available and the application being used determines the array and the associated hardware.

The capillary array (Figure 2) has three components: Electrode Block (inlet), eight capillaries and the Array Fitting (outlet).

- The Electrode Block is the DNA sample inlet side of the array. It holds eight hollow, stainless-steel electrodes. Each stainless-steel electrode holds a capillary in the center. These electrodes are designed such that they can be immersed into an entire row (8 wells) of a 96-well microplate (8-rows x 12-columns).
- The capillaries pass through and exit the Array Fitting. When the fitting is installed, the ends of the capillaries are submerged in the Gel/Buffer Manifold Reservoir.
- The Array Fitting contains the Detection Window and is the outlet side of the array. The Detection Window of the fitting is used to expose the eight capillaries to laser excitation. (The external polyimide coating of the capillaries has been removed for this purpose.)

When the capillary array is not installed, e.g., during shipping and storage, the Manifold Plug is inserted into the Manifold (Array Fitting outlet) to prevent any gel (inside of the CEQ) from drying.

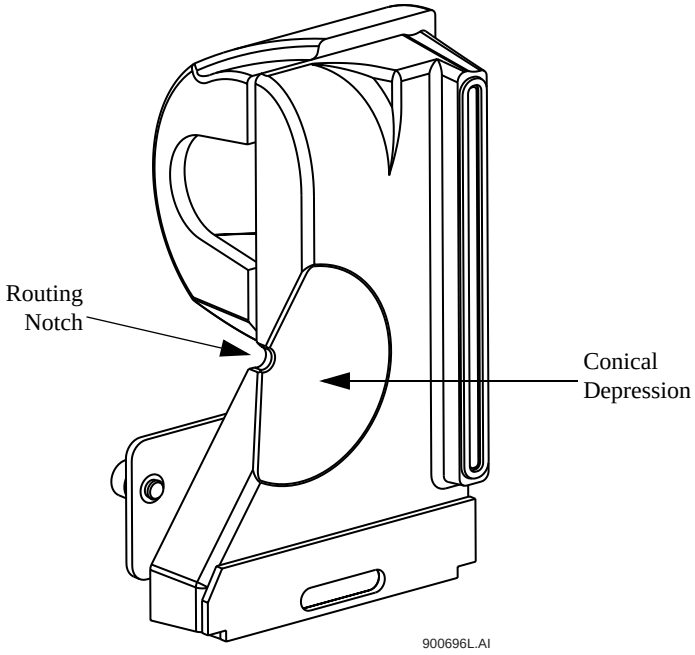
Figure 2: Capillary Array



Plenum

Due to the differences in the length of the capillary arrays used for various sequencing and fragment applications, the capillary heater plenums also differ. When looking at the capillary heater plenums, two differences can be noted. First, the Short Plenum Assembly, used with the shorter capillary array, has a capillary array routing notch and a conical depression on the back side (Figure 3). This allows the shorter capillary array to pass through the notch in the Plenum without constricting any of the capillaries. The Long Plenum Assembly does not have either of these features. Second, the Caution label on the front of the each plenum specifies the length of the capillary array to be used with that particular plenum.

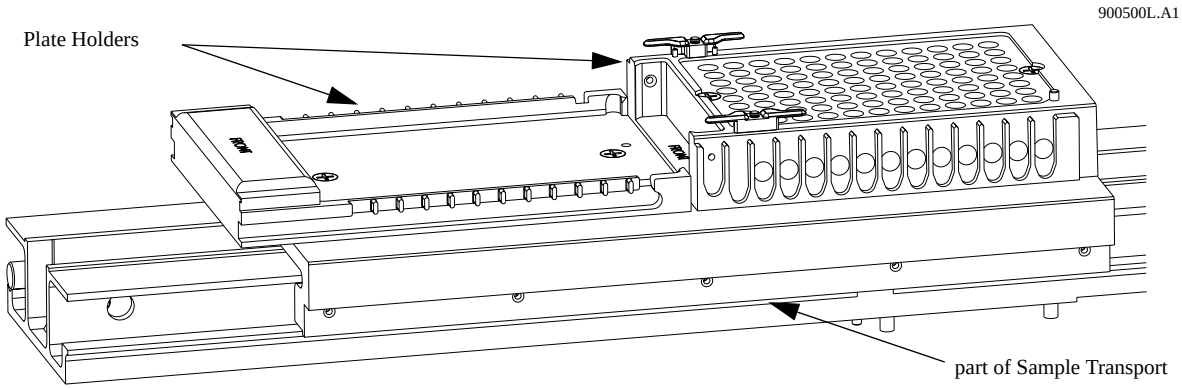
Figure 3: Short Plenum Assembly (Back View)



Sample Transport and Plate Holders

The Sample Transport (Figure 4) contains the Plate Holders which are used to hold the Sample Plate, Buffer Plate w/Buffer Evaporation Cover and Wetting Tray.

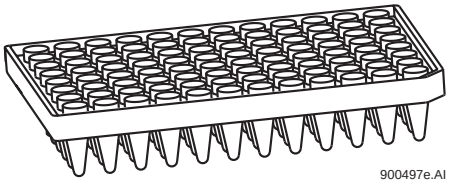
Figure 4: Sample Transport and Plate Holders



Sample Plate

The Sample Plate (Figure 5) is used to hold the sample(s) for separation. The plate is a V-bottom, thermal cycler-compatible, polypropylene plate containing 96 wells (8-rows x 12-columns). The wells have a 200µL volume capacity.

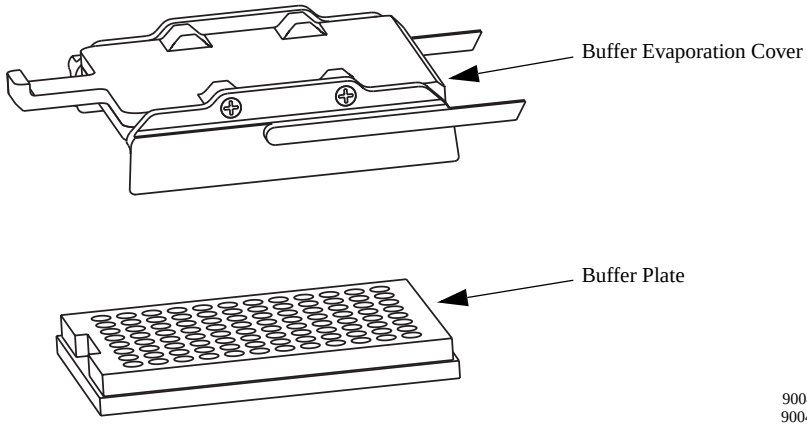
Figure 5: Sample Plate



Buffer Plate with Buffer Evaporation Cover

The Buffer Plate (Figure 6) is used to hold the DNA separation buffer used during a sample run. The plate is a flat-bottom, polystyrene, non-sterile plate containing 96 wells (8-rows x 12-columns). An evaporation cover, placed over the Buffer Plate, is used to maintain the proper level of buffer (250-300 µL) in the Buffer Plate by preventing dissipation of the buffer. The cover slips over the Buffer Plate position of the Sample Transport. As the CEQ advances through the Sample Plate during a run, the evaporation cover is pushed back just far enough to expose the next row of buffer wells to be used for the next sample set run.

Figure 6: Buffer Evaporation Cover and Buffer Plate



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Wetting Tray

The Wetting Tray (Figure 7) is used to immerse the ends of the capillaries in deionized water. When properly filled, the capillaries can be maintained for approximately seven days in the Wetting Tray without attention. During continuous use of the CEQ 2000 System, the Wetting Tray should be replenished with deionized water after a 96-well microplate has been run or prior to a sample plate run. The wetting tray may also be used to rinse capillary tips or provide a receptacle for separation medium purged from the capillaries.

CAUTION **No more than one 96-well plate should be processed without replenishing the Wetting Tray. For information on Cleaning and Filling the Wetting Tray, see page 4-6.**

Figure 7: Wetting Tray

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Gel Waste Bottle

The Gel Waste Bottle (Figure 8) is used to capture and store the waste gel that is pushed out of the manifold during the purge function. The bottle can hold the equivalent of 25 gel cartridges. Periodic observation of the gel level in the waste bottle is recommended to prevent overflow.

Figure 8: Gel Waste Bottle

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Power Switch

This push-button switch, when in the **ON** position, provides power for the CEQ.

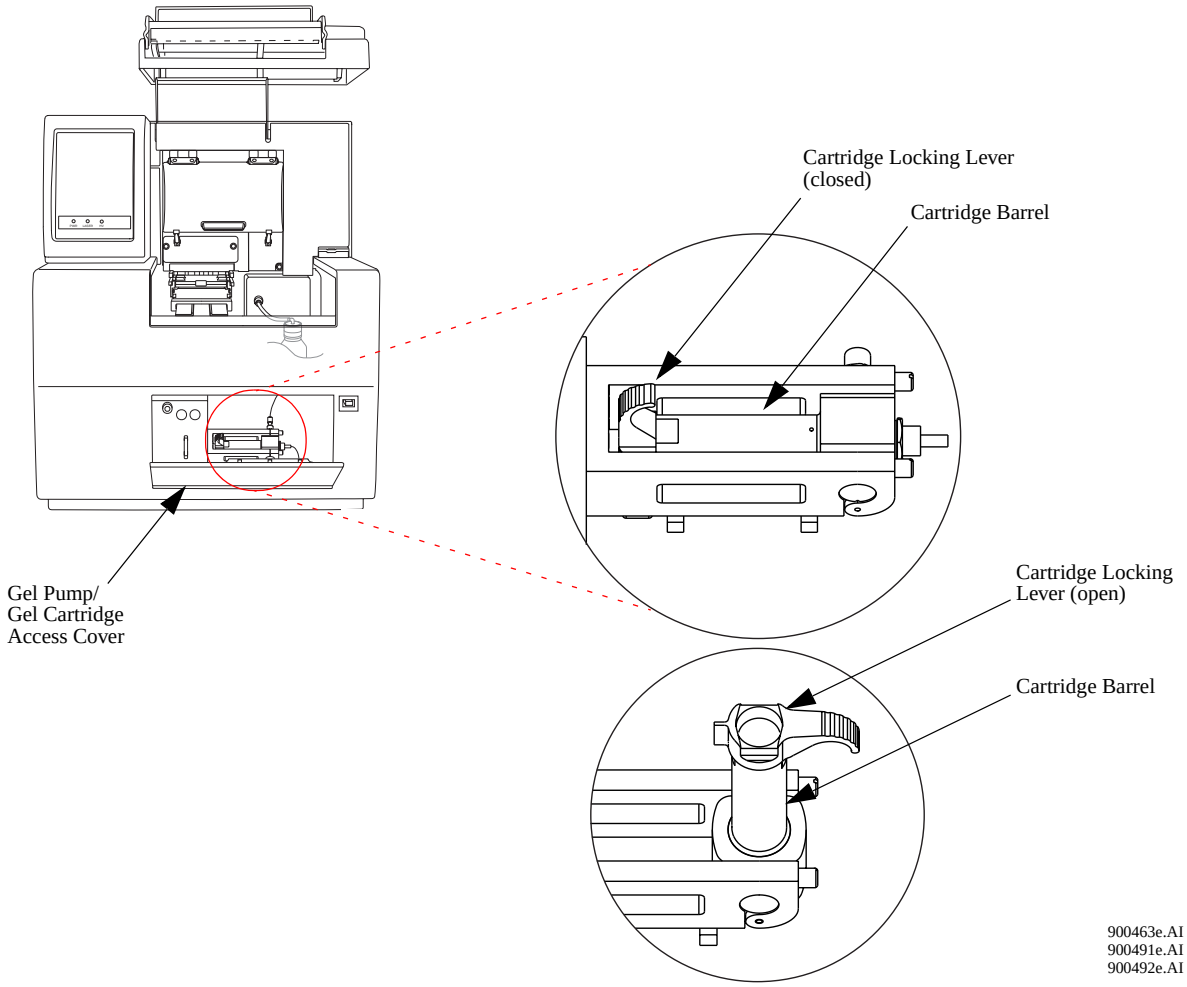
Gel Pump/Gel Cartridge Access Cover

Provides access to the Gel Pump and Gel Cartridge.

Gel Pump, Gel Cartridge and Gel Pump Plug

Gel Pump (Figure 9)	Used to replenish the capillaries with fresh gel (from the cartridge) after each sample set and each 96-well plate run.
Gel Cartridge	Contains the fresh DNA separation gel. The separation gel is used for both Sequence and Fragment Analysis. When full, the cartridge contains a sufficient amount of gel for 12 runs (96 templates). (See Table 64 on page 4-12.)
Gel Pump Plug	When the gel cartridge is not installed, e.g., during shipping and storage, the Gel Pump Plug is inserted into the pump barrel to prevent gel from drying in the system.

Figure 9: Gel Pump



Status Indicator Lights

There are three status indicators: PWR (power), LASER and HV (high voltage).

PWR	Green when power is supplied to the CEQ. Off when no power is supplied.
LASER	Green when the lasers are turned on during a run. Off when the lasers are turned off.
HV	Green when the high voltage is applied during the injection or separation. Off when the high voltage is turned off.

Software

The software provides the interface for manual or automatic (pre-programmed) control of the system and for data capture and basic data analysis.

User Interface

The Main Menu is shown in Figure 10. This window provides access to all modules of the CEQ 2000 Software. The software modules are described in Table 1.

Figure 10: Main Menu

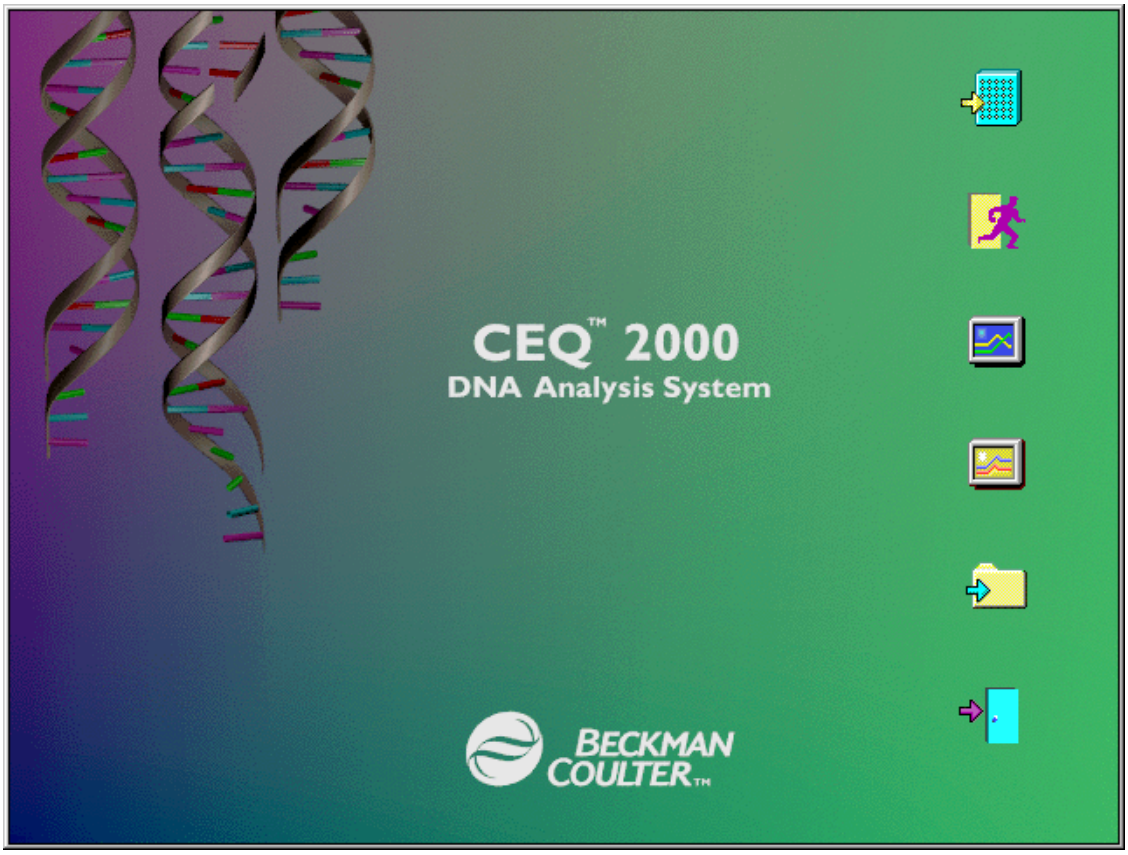
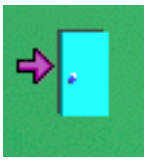


Table 1: Software Module Descriptions

Module	Description
	***Sample Setup Module*** Use this module to create, save and modify sample plates. Sample plates are used to assign methods to control sample sets and determine the sequence of methods that will be used to produce data.
	***Run Module*** Use this module to run sample plates and control individual functions of the instrument.
	***Sequence Analysis Module*** Use the Sequence Analysis module to view, analyze, compare, manipulate and print base sequence data produced by sample runs.
	***Fragment Analysis Module*** Use the Fragment Analysis module to view, analyze, compare, manipulate and print fragment data produced by sample runs.
	***Data Manager Module*** Use this module to create, modify and print databases items.
	***Exit*** When selected, the Exit icon closes any active modules and shuts down the CEQ 2000 system.

Safety Information

This section provides safety information and instructions for the hardware and accessories of the system. It is broken down into the following subsections:

- Symbols
- Safety Features
- Chemical and Biological Safety
- Electrical Safety
- Moving Parts Safety
- Laser Safety
- Electrostatic Discharge

Safety Symbols

The symbols displayed below and on the instrument are reminders that all safety instructions should be read and understood before installation, operation, maintenance or repair to this instrument is attempted.

When symbols are displayed in this manual, pay particular attention to the safety information associated with the symbol.

WARNING

This icon accompanies text and/or other international symbols dealing with hazards to personnel. When present, it indicates that a potential hazard to your personal safety exists if information stated within the "**WARNING**" paragraph is not adhered to or procedures are executed incorrectly.

CAUTION

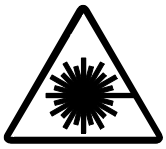
This icon accompanies text and/or other international symbols dealing with potential damage to equipment. When present, it indicates that there is a potential danger of equipment damage, software program failure or that a loss of data may occur if information stated within the "**CAUTION**" paragraph is not adhered to or procedures are executed incorrectly.

HIGH VOLTAGE

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a high voltage source. In this document, the "**WARNING**" icon will accompany this symbol.

BIOHAZARD

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a biological source. In this document, the "**WARNING**" icon will accompany this symbol.



LASER LIGHT

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a laser source. In this document, the "**WARNING**" icon will accompany this symbol.

SHARP OBJECTS

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from unblunted corners or other appendages on the outside or inside of the equipment. In this document, the "**WARNING**" icon will accompany this symbol.

HOT SURFACE

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from heated faces or other appendages on the outside or inside of the equipment. In this document, the "**WARNING**" icon will accompany this symbol.

CONFORMILE EUROPEENE

Equipment displaying the CE mark has been tested and is in compliance with all applicable European safety directives.

Protective Earth or Ground Terminal

This symbol identifies the location of the protective earth or ground terminal lug on the equipment.

OFF Position of Principal Power Switch

This symbol graphically represents the equipment main power *rocker* switch when it is in the off position.

ON Position of Principal Power Switch

This symbol graphically represents the equipment main power *rocker* switch when it is in the on position.

Safety Features

Review the location and action of the following safety features:

- ON/OFF Switch
- High Voltage Interlock
- Capillary Access Cover

Chemical and Biological Safety

WARNING **Normal operation of the system can involve the use of solvents and reagents that are toxic, flammable or biologically harmful.**

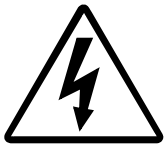
- **Observe all precautionary information printed on the original solution containers.**
- **Operate the system in the appropriate environment.**
- **Take all necessary precautions when using pathology or toxic materials to prevent the generation of aerosols.**
- **Observe all applicable precautionary procedures when using flammable solvents in or near the instrument.**
- **Wear appropriate laboratory attire, e.g., safety glasses, gloves, lab coat and breathing apparatus, when working with hazardous materials.**
- **Dispose of all waste solutions in a proper manner.**

Electrical Safety

To reduce risk of electrical shock, all devices employ a three wire electrical cable and plug to connect the equipment to earth ground.

- Ensure that the wall outlet receptacle is properly wired and earth grounded.
- DO NOT use a three-to-two wire plug adapter.
- DO NOT use a two wire extension cord or a two wire multiple-outlet power strip.
- Disconnect power to the system before performing maintenance.
- DO NOT remove any panels; panels should be removed only by qualified service personnel.

WARNING **A high voltage power supply is used with this instrument. Safety interlocks disable high-voltage output while the cartridge cover is open and remove the risk of shock while performing routine instrument functions. However, removal of any panel may expose an individual to the possibility of severe electrical shock and/or mechanical injury. For this reason, any service requiring removal of a panel or otherwise overriding or disabling safety interlocks must be done by Beckman Coulter personnel only.**



Moving Parts

Moving parts are limited to the sample handling system. Plate movement is safety interlocked through the Capillary Access Cover. To avoid injury due to moving parts, observe the following:

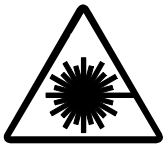
- Keep loose clothing and hair away from the plate area.
- Never attempt to physically restrict movement of the plate assembly.

Electrostatic Discharge

CAUTION **Always properly ground yourself before working with the system electronics. Contact Beckman Coulter Field Service if you have questions.**

Laser Safety

WARNING **The CEQ System uses a "Class 3B" laser. The "3B" classification means that "direct intrabeam viewing of this type of laser is always hazardous to personnel."**

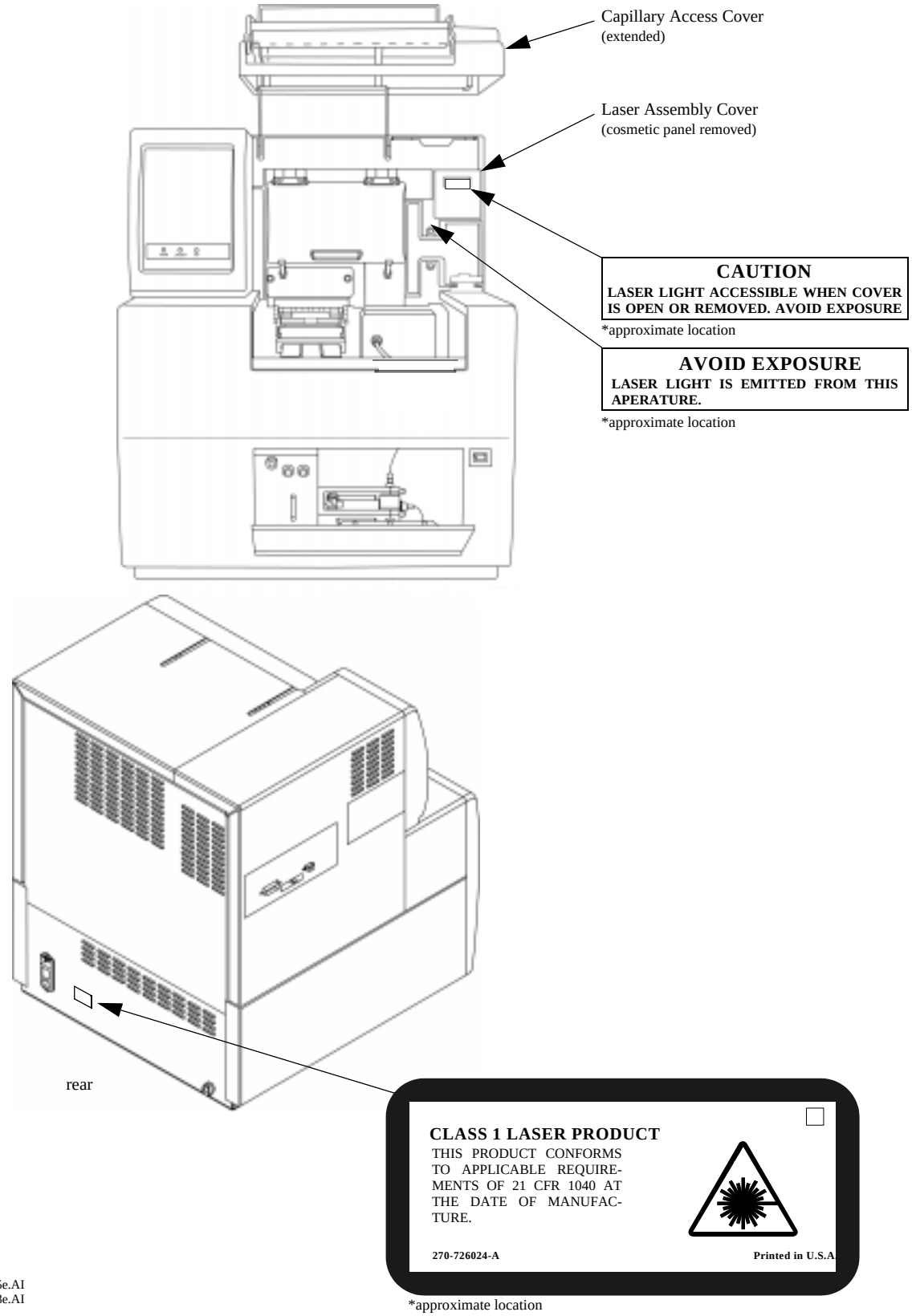


The laser (and several other integral components) are housed in a sealed container that together comprise the Laser Assembly. The Laser Assembly has no user serviceable parts. Service of the Laser Assembly is restricted to certified Beckman Coulter Field Engineers.

During normal operation of the system, laser light is not accessible to the user. Therefore, the overall laser classification of the CEQ System is "Class 1," i.e., "lasers which are safe under reasonably foreseeable conditions of operation."

To prevent users from potentially harmful laser light, observe all safety warnings (see Figure 11 for label locations) and NEVER REMOVE THE OUTER CASING OF THE LASER ASSEMBLY.

Figure 11: Laser Label Locations



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Chapter Two 2

Program Description

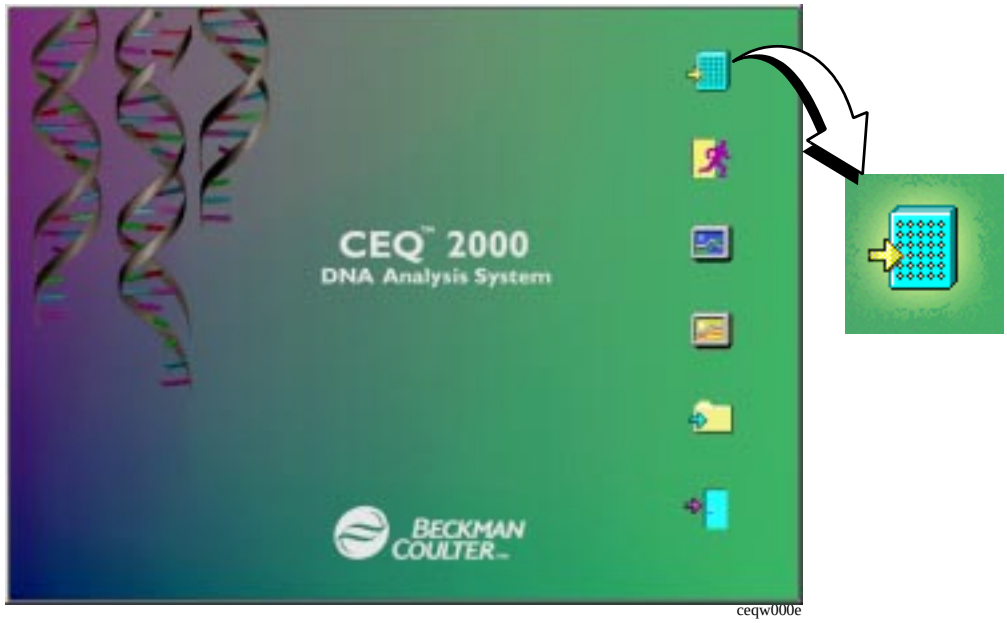
Chapter Overview

This chapter provides descriptions of the main windows, menus and dialogs controlling the system.

Sample Setup Module

The Sample Setup module is used to create, save and modify methods and sample plates. Sample plates are the means by which samples are named and organized. Methods consist of sequential events needed to perform a DNA separation. The sample plate definition contains the 96-well plate locations of the samples as well as410 the method assigned to each sample. The capillary array must run the same parameters across eight samples, one column at a time. Each column that contains samples is called a "sample set." The separation parameters applied to each sample set are specified in the "method." You can also edit method parameters to modify or create new methods. Selecting the Sample Setup *icon* from the Main Menu (Figure 12) executes this module.

Figure 12: Main Menu, Sample Setup Icon



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If the Shortcut Bar (Figure 13) is displayed, the Sample Setup *icon* accesses this module.

Figure 13: Shortcut Bar Icons, Sample Setup

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Main Window

The main window of this module is shown in Figure 14 and described in Table 2.

Figure 14: Sample Setup Module, Main Window

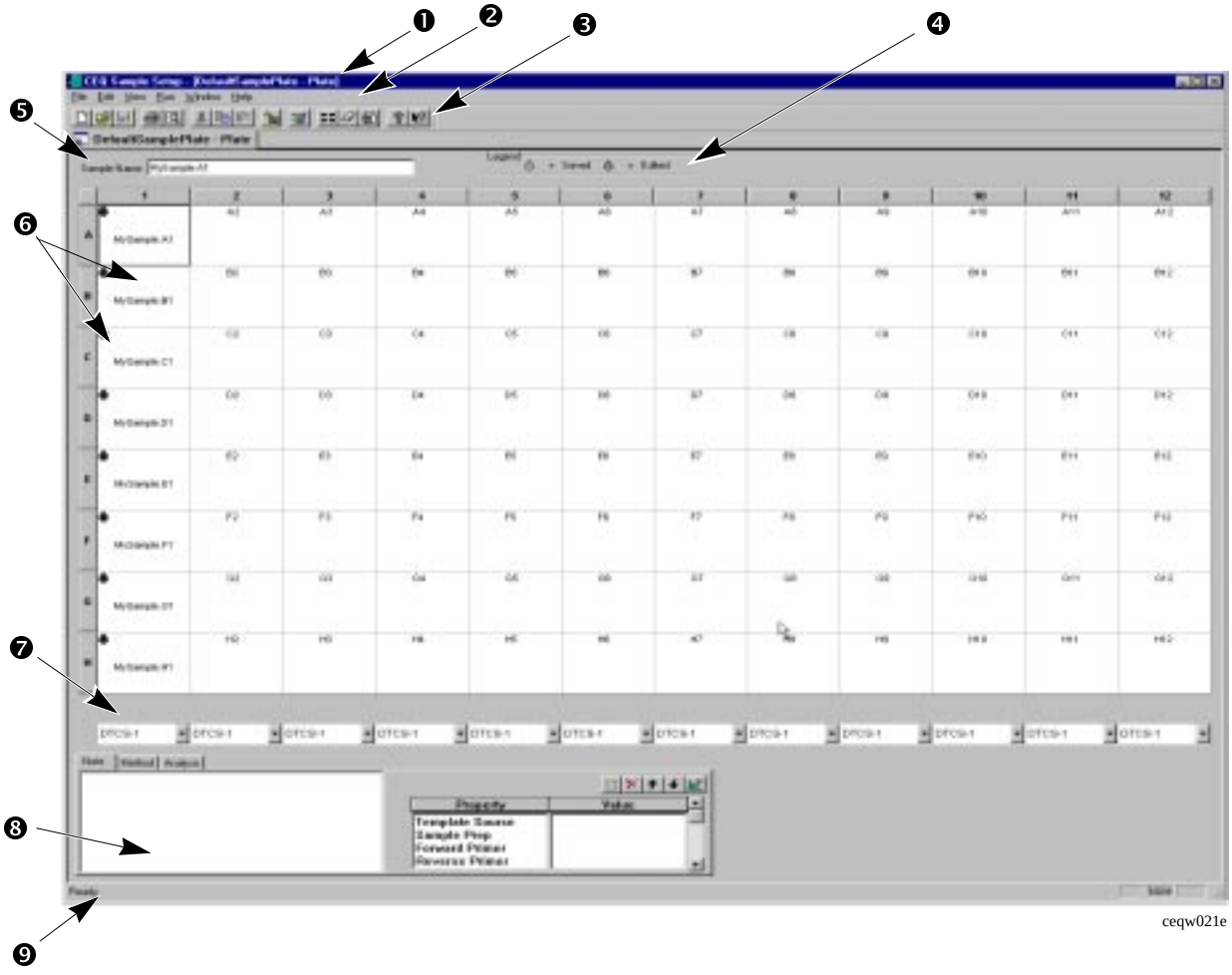

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Table 2: Sample Setup Module, Main Window Descriptions

Item	Description
❶	The ***Title Bar*** showing the module name ("*CEQ Sample Setup*") and the active sample plate ("*DefaultSamplePlate*").
❷	***Menu Bar*** (See ***"Menu Bar Options"*** on page 2-6.)
❸	***Toolbar*** (See ***"Toolbar Icons"*** on page 2-10.)
❹	***Legend*** showing the color coding information (Saved or Edited) of the individual cells.
❺	***Sample Name*** of the currently active cell.
❻	***Sample Plate*** containing 96 cells that corresponds to a 96-well sample plate. (Sample plate ***Columns*** are numbered 1 through 12 and ***Rows*** are labeled A through H. This naming convention uniquely identifies each sample location.)
❼	Currently active (selected) ***Method***. This drop-down is used to assign a pre-defined method to the corresponding samples in the column above. • The **DTCS** methods are used for standard sequencing. • The **Frag** methods are used for fragment analysis. • The **Condition** method is used to condition a new capillary array. • The **LFR** method is used for long fast read sequencing.

Table 2: Sample Setup Module, Main Window Descriptions

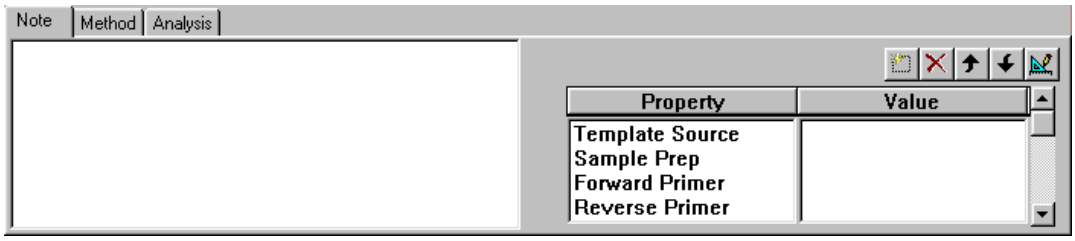
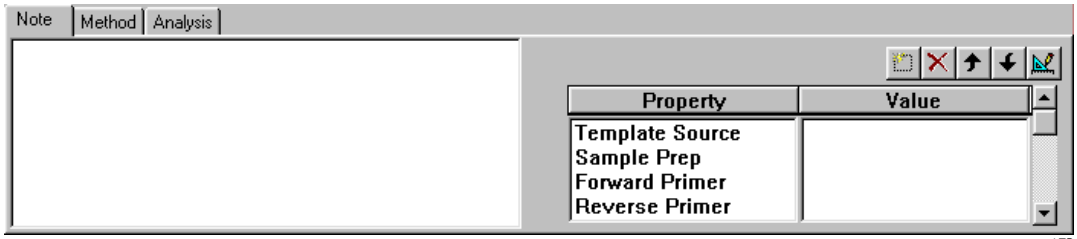
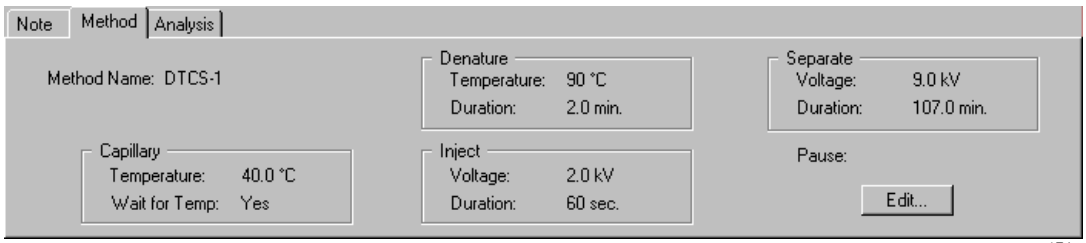
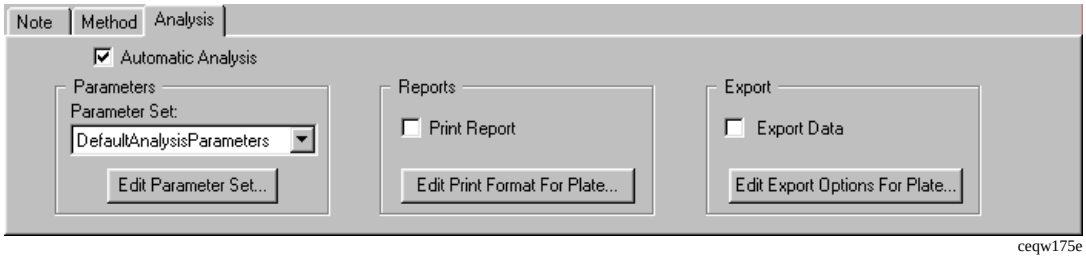
Item	Description
❽	***Notes Window*** Used to enter explanatory text concerning the selected sample(s). The text box is blank if multiple samples *with different descriptions* are selected. **Figure 15: Notes Window**  ceqw173e ***Property Sets Listbox*** Property sets are groups of properties that can be applied to a sample(s), such as Vector, Host and Primer used. The software provides three templates: Cloning, PCR and FLP. **Figure 16: Property Sets List Box**  ceqw173e  Used to create a new property.  Used to delete a property.  Used to move a property up in the properties list.  Used to move a property down in the properties list.  Used to create or edit a property set.

Table 2: Sample Setup Module, Main Window Descriptions

Item	Description
❽ (cont'd)	***Method Window*** Provides a summary of the separation parameters defined for the selected method. Edit existing or create a new method by clicking on the **Edit** button. **Figure 17: Method Window**  ceqw174e ***Analysis Window*** Used to: • Enable/disable the **Automatic Analysis** mode. When enabled, this mode causes the CEQ to automatically analyze the sample set after it has run, using the Analysis Parameter Set selected in the drop-down menu. • Choose and/or edit Sequence Analysis or Fragment Analysis **Parameter Sets** via the drop-down menu. • Enable/disable automatic printing of reports, view and/or edit the print report options. • Enable/disable automatic exporting of data, view and/or edit export options. **Figure 18: Analysis Window**  ceqw175e
❾	The ***Status Bar*** displays information concerning the current selection.

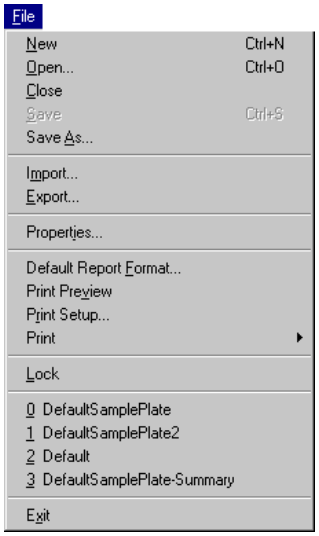
Menu Bar Options

The menu bar has the options shown in Figure 19. Tables 3 through 8 provide descriptions of the menu options.

Figure 19: Sample Setup Module, Menu Bar Items

ceqw139e

File Menu

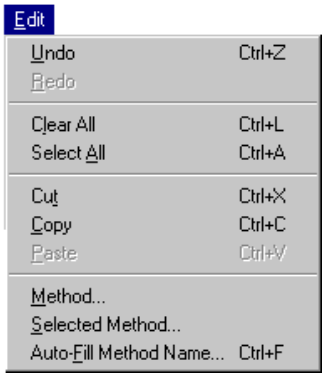


ceqw022e

Table 3: Sample Setup Module, File Menu

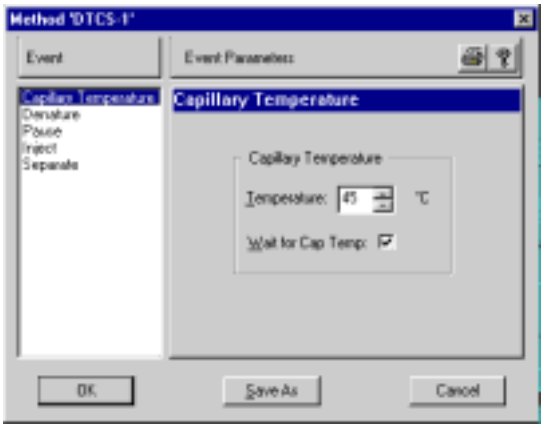
Item	Description
New	Used to open an empty sample plate.
Open	Used to select an existing sample plate to open.
Close	Used to close the selected sample plate.
Save	Used to save the current sample plate.
Save As	Used to save the sample plate with a new name.
Import	Used to import a sample plate (Tab Delimited ASCII Text [*.txt] format).
Export	Used to export a sample plate (Tab Delimited ASCII Text [*.txt] format).
Properties	Provides an information window concerning the active sample plate.
Default Report Format	Used to define the report formats for new and imported sample plates.
Print Preview	Used to display a facsimile of a hardcopy printout of the active sample plate.
Print Setup	Used to define printer properties.
Print	Pull-right menu used to print a detailed report of the active sample plate, the contents of the sample plate, the plate summary or the selected method.
Lock	Used to prevent editing of the selected plate.
Exit	Used to close the Sample Setup module.

Edit Menu



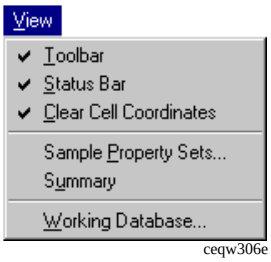
ceqw025e

Table 4: Sample Setup Module, Edit Menu

Item	Description
Undo	Used to undo the last action performed.
Redo	Used to redo the last undo action.
Clear All	Used to empty all cell properties in the sample plate.
Select All	Used to select all cells in the sample plate.
Cut	Used to delete text or the applied properties in one or more cells of the sample plate.
Copy	Used to duplicate text or the applied properties in one or more cells of the sample plate.
Paste	Used to insert copied or cut items at the cursor insertion point.
Method	Used to select a method to edit.
Selected Method	Used to view or edit the currently selected method from the dialog box shown below.  ceqw027e
Auto-Fill Method Name	Used to select a method from the drop-down menu to be used with the selected sample set of the plate or all sample sets in the plate.  ceqw305e

View Menu

Table 5: Sample Setup Module, View Menu



ceqw306e

Item	Description
Toolbar	Toggles between displaying/not displaying the toolbar.
Status Bar	Toggles between displaying/not displaying the Status Bar.
Clear Cell Coordinates	Toggles between displaying/not displaying the coordinates of the plate cells.
Sample Property Sets	Used to set up the property set for the selected sample(s).
Summary	Displays a summary of the currently selected plate.
Working Database	Displays a dialog box showing the database in use.

Run Menu

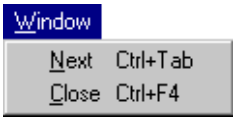
Table 6: Sample Setup Module, Run Menu

ceqw308e

Item	Description
Start	Invokes the Run module and loads the active sample plate.

Window Menu

Table 7: Sample Setup Module, Window Menu

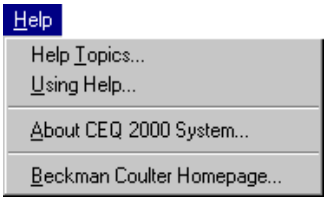


ceqw309e

Item	Description
Next	Toggles between open sample plates.
Close	Used to close the active sample plate.

Help Menu

Table 8: Sample Setup Module, Help Menu



ceqw310e

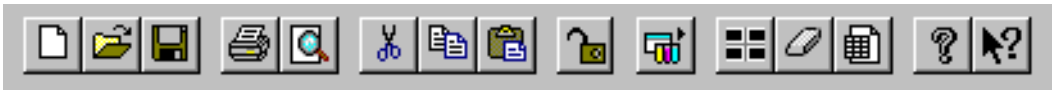
Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.

Item	Description
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ 2000 System	Used to access software, instrument and system information.
Beckman Coulter Homepage	Used to access the Beckman Coulter Homepage on the Internet.

Toolbar Icons

The toolbar (Figure 20) contains icons that correspond to common menu options. The information in Table 9 describes the function of each icon.

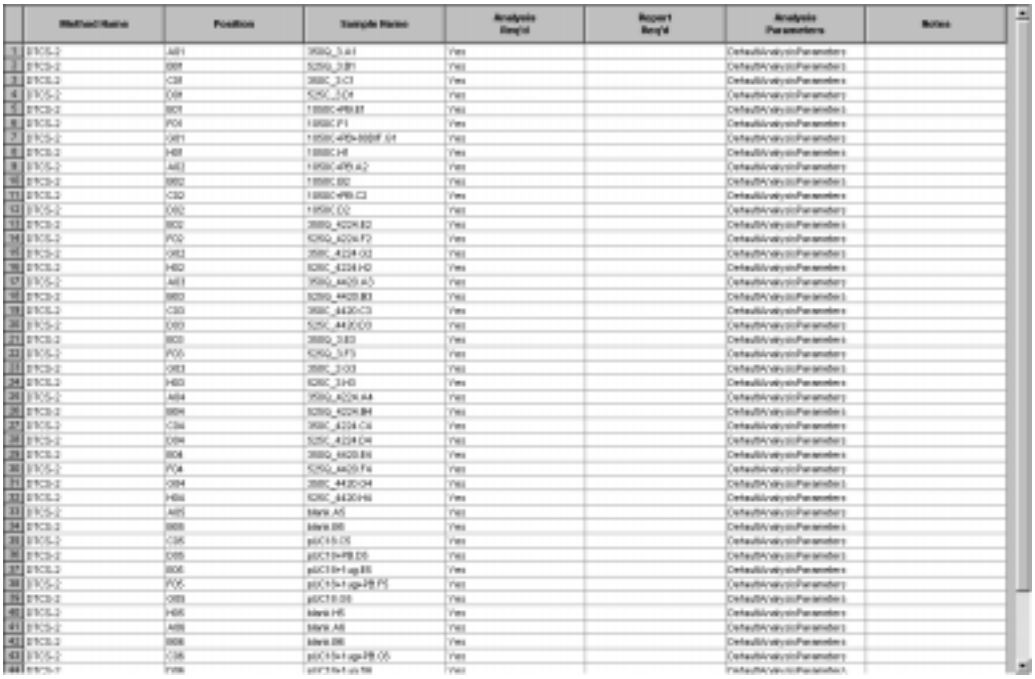
Figure 20: Sample Setup Module, Toolbar



ceqrf104

Table 9: Sample Setup Module, Toolbar

Icon	Description
	***New*** - Used to open an empty sample plate.
	***Open*** - Used to select an existing sample plate to open.
	***Save*** - Used to save the current sample plate.
	***Print Sample Plate*** - Used to print a detailed report of the active sample plate.
	***Print Preview*** - Used to display a facsimile of a hardcopy printout of the active sample plate.
	***Cut*** - Used to delete text in one or more cells of the sample plate.
	***Copy*** - Used to duplicate text in one or more cells of the sample plate.
	***Paste*** - Used to insert copied or cut items at the cursor insertion point.
	***Unlock*** - Indicates that the selected sample plate is available for editing. Clicking on the icon locks the plate to prevent editing and changes the icon to  .
	***Run*** - Invokes the Run module and loads the active sample plate.
	***Select All*** - This button is used to highlight (select) all cells in the sample plate.
	***Clear All*** - This button is used to remove all cell properties in the sample plate.

Icon	Description
	***View Summary*** - View a summary of a specific sample plate by opening the desired sample plate and clicking on the View Summary icon. The Sample Plate Summary spreadsheet is displayed with a summary of each sample in the plate. This is a read-only spreadsheet and cannot be edited. **Figure 21: Sample Plate Summary** 
	***Help Index*** - Used to select and print specific topics in the CEQ Help file and search for information by: topic, index entry and/or keyword.
	***Context-Sensitive Help*** - Used to open the Help file related to a specific menu option.

Run Module

The Run module provides the capability of executing pre-programmed sample plates and controlling individual functions of the instrument. Specifically, it is used for:

- Running and viewing the progress of a sample plate
- Viewing and changing display options
- Specifying a sample set, buffer set or wetting tray position
- Specifying the capillary holding temperature
- Denaturing a sample
- Aligning the capillaries
- Injecting a sample
- Performing a separation
- Changing plates, wetting tray, capillary array or gel cartridge

Selecting the Run *icon* from the Main Menu (Figure 12) executes this module.

Main Window

The main window of the Run module is shown in Figure 22 and described in Table 10. Tables 11 through 18 provide descriptions of the menu options.

Figure 22: Run Module, Main Window

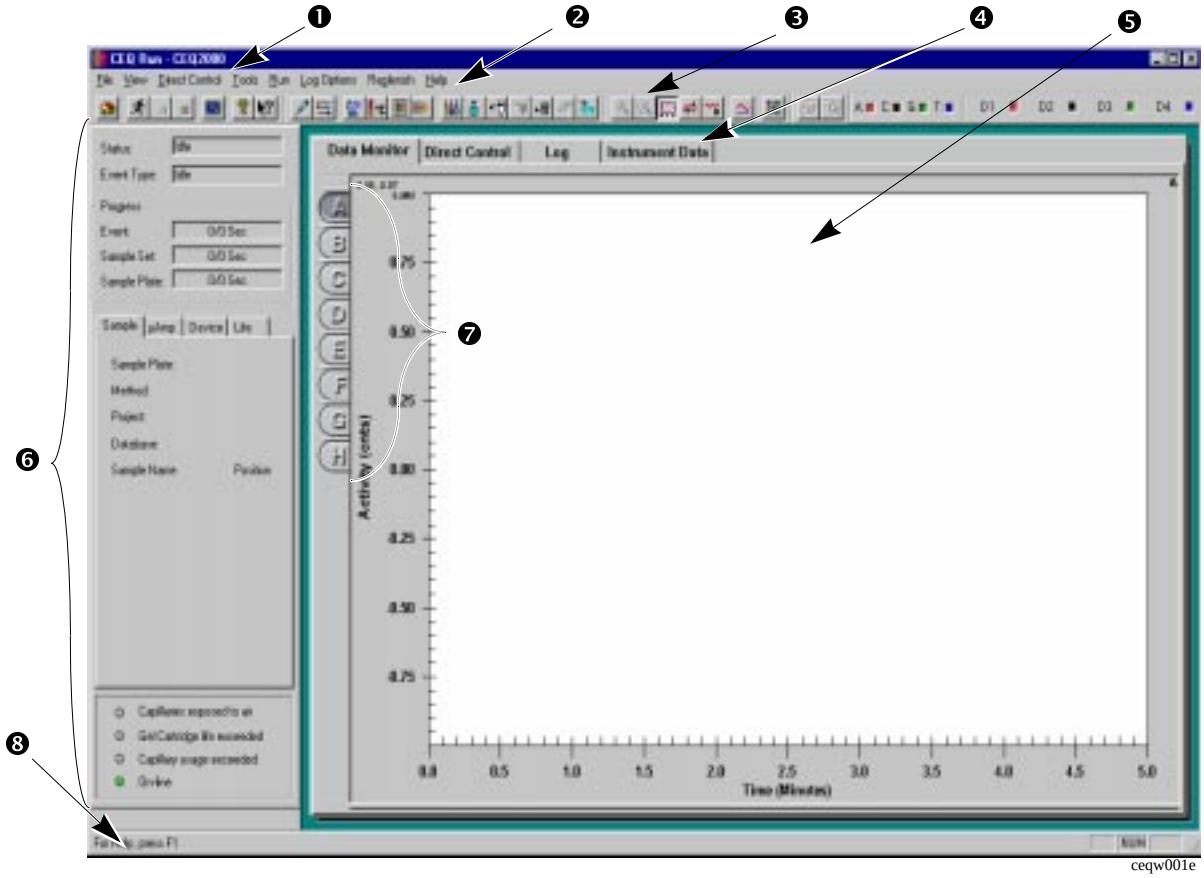


Table 10: Run Module, Main Window Descriptions

Item	Description
❶	The ***Title Bar*** displays the name of the module (*CEQ Run*) and the system name (*CEQ 2000*).
❷	The ***Menu Bar*** is a list of the menu options (see ***"Menu Bar Options"*** on page 2-14).
❸	The ***Toolbars*** contain the icons that execute pre-defined functions (see ***"Toolbar Icons"*** on page 2-26).
❹	The ***Window Selection Tabs*** are used to toggle between windows (see ***"Window Selection Tabs"*** on page 2-32).
❺	The ***Display Area*** graphically displays the raw data of the selected capillary or capillaries.
❻	The ***Status Monitor*** provides information concerning the current state of the run, capillary and gel cartridge status and system on-line/off-line status.
❼	***Capillary Buttons*** - Letters **A** through **H** represent the eight capillaries in the array. Each button selected will have an associated pane shown in the Display Area.
❽	The ***Status Bar*** is used to display messages.

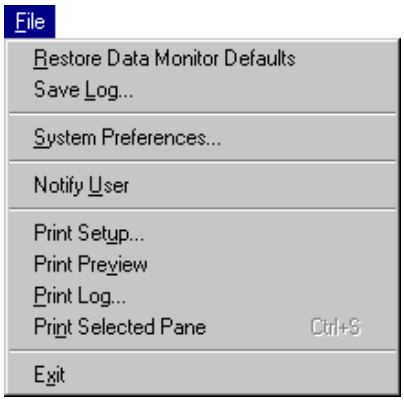
Menu Bar Options

The menu bar has the options shown in Figure 23. Tables 11 through 18 describe the options.

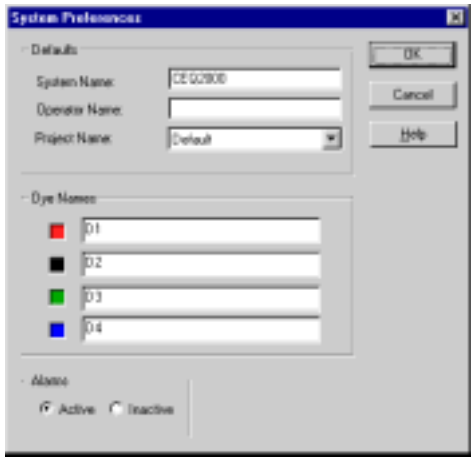
Figure 23: Run Module, Menu Bar Items

File Menu

Table 11: Run Module, File Menu

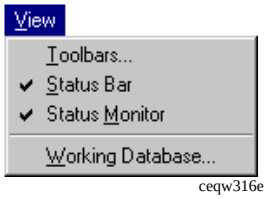


ceqw314e

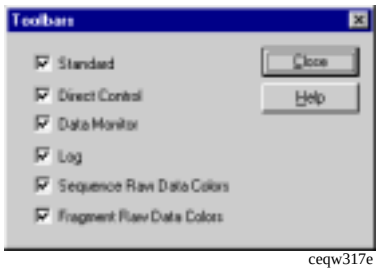
Item	Description
Restore Data Monitor Defaults	Restores the colors of the data monitor to the original default shipped with the instrument.
Save Log	Used to select the folder where the system log will be stored. The log is identified by the date and a unique identifier.
System Preferences	Used to view and/or change the: **System Name**, **Operator Name**, **Project Name** and **Dye Names** for Fragment Analysis and/or to activate or deactivate the alarms.  ceqrf228
Print Setup	Used to define printer properties.
Print Preview	Used to display a facsimile of a hardcopy printout of the System Log prior to printing.
Print Log	Used to print the System Log.
Print Selected Pane	Used to print a single pane selected in the Display Area.
Exit	Closes the Run module.

View Menu

Table 12: Run Module, View Menu

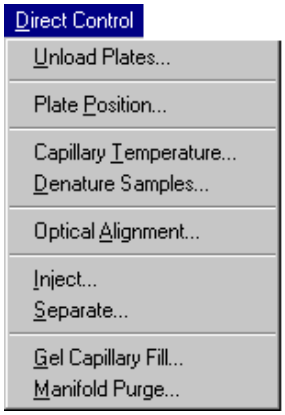


ceqw316e

Item	Description
Toolbars	Used to select the toolbars to display in the Run module window.  ceqw317e
Status Bar	Toggles between displaying/not displaying the Status Bar.
Status Monitor	Toggles between displaying/displaying the Status Monitor (see ***"Status Monitor"*** on page 2-31).
Working Database	Displays a dialog box showing the database in use.

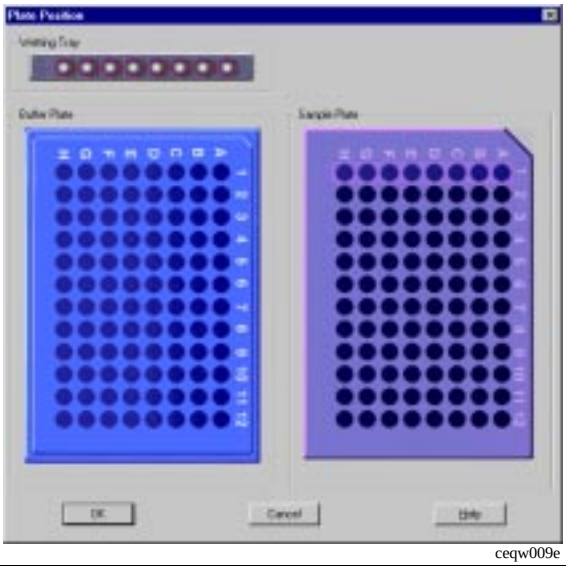
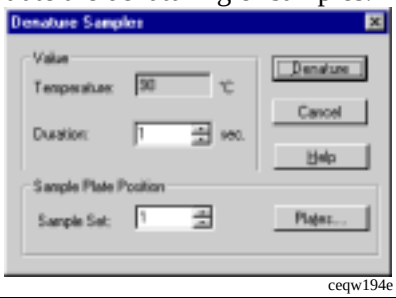
Direct Control Menu

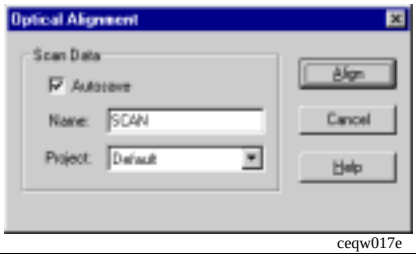
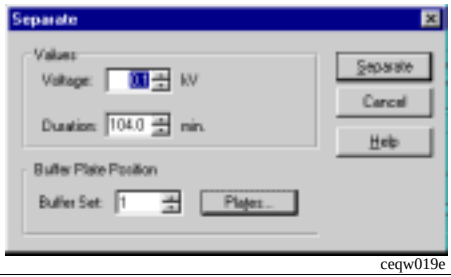
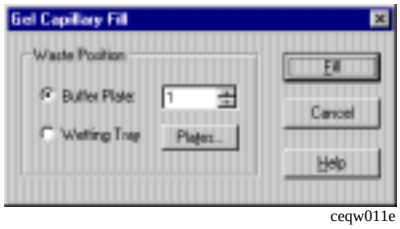
Table 13: Run Module, Direct Control Menu



ceqw012e

Item	Description
Unload Plates	Used to: • select the position for capillary immersion in the plate or wetting tray. • move the plates to the Load position at the front of the instrument to load or unload plates.  ceqrf302

Item	Description
Plate Position	Used to select the plate or tray position where the capillaries will be immersed.  ceqw009e
Capillary Temperature	Used to change the capillary holding temperature.  ceqw013e Note ***This temperature will be over-ridden by the purge cycle at the end of each sample plate and by the shutdown method performed when a sample plate is cancelled.***
Denature Samples	Used to initiate the denaturing of samples.  ceqw194e

Item	Description
Optical Alignment	Used to align the lasers with the detection windows of the eight capillaries. Data can be saved and viewed in the Sequence and Fragment Analysis modules.  ceqw017e
Inject	Used to initiate the injection of the sample prior to separation.  ceqw018e
Separate	Used to initiate the separation of injected samples.  ceqw019e
Gel Capillary Fill	Used to fill all capillaries with fresh gel. (Any gel present in the capillary is forced out to the specified waste position in the buffer plate or wetting tray.)  ceqw011e

Item	Description
Manifold Purge	Used to clear the manifold of air pockets and/or remaining DNA fragments before the next separation process.  ceqw005e

Tools Menu

Table 14: Run Module, Tools Menu

ceqw209e

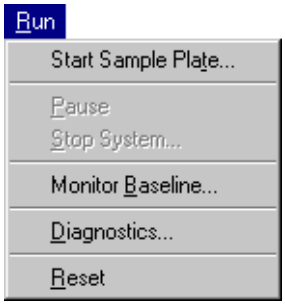
Item	Description
Autoscroll	Used to scale all data to the last 10 minutes of the run on the X axis and confine the display of data to the pane on the Y axis.
Autoscale	Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this item to turn autoscaling on or off.
Pause Data Display	Used to pause the display of data. Pausing data does not stop the stream of data, just the display of data.
Unzoom	Used to undo one zoom level.
Unzoom All	Used to undo all zoom levels.

Table 14: Run Module, Tools Menu

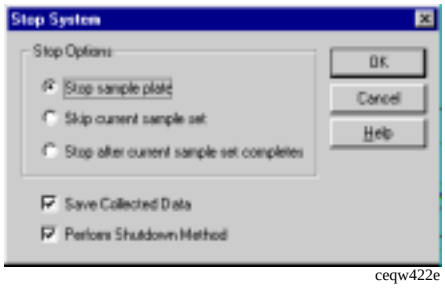
Item	Description
Display Options	Invokes the **Display Options** dialog box used to modify any or all of the following parameters: • Title • X Axis Options • Y Axis Options • Dye Traces • Current Traces • Colors See immediate changes in the selected pane by clicking on **Apply**.  ceqw1094e ***Double-clicking the left mouse button in the selected pane, also brings up this dialog box.***
View Last Analysis	Used to open the Analysis module and display the most recently analyzed sample set. If the last sample set was analyzed with a sequence analysis parameter set, the Sequence Analysis module is launched. If the last sample set was analyzed with a fragment analysis parameter set, the Fragment Analysis module is launched.

Run Menu

Table 15: Run Module, Run Menu



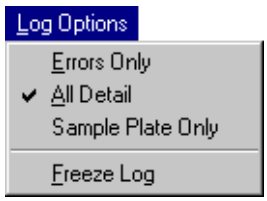
ceqw239e

Item	Description
Start Sample Plate	Used to open and execute a specific sample plate.
Pause	Used to pause a running sample plate.
Stop System	Used to stop a running sample plate or direct control process.  ceqw422e **Stop Options -** Specifies when to stop sample plate: **Stop sample plate** at the end of the currently executing step, **Skip current sample set** (currently executing sample set), ***OR*** **Stop after current sample set completes** (at the end of the currently executing sample set). **Save Collected Data:** Save data already collected for the currently executing sample set. **Perform Shutdown Method:** Purge the system of any remaining DNA fragments and refill the capillaries with fresh gel after the sample plate has been cancelled. (The capillary temperature will be set to 40°C.)

Item	Description
Monitor Baseline	Baseline monitoring displays the baseline data trace. To ensure that the optics are working correctly and that the capillaries are clean, view the baseline when a new capillary array is installed and after the capillaries have been filled with gel for the first time. The baseline trace should be low and relatively flat. If **Autosave** is selected from the dialog box, baseline data will be stored under the Sample Data node (for the selected project) in the Data Manager. If Monitor Baseline is not ended manually, baseline data will be collected until a sample plate is run or until six hours have elapsed from the start of the baseline monitoring process.
Diagnostics	Used to view the instrument version, laser power, PC Settings, monitor status, and to home the plates and/or the gel pump.
Reset	Used to re-synchronize system hardware with the firmware and to reset the system log.

Log Options Menu

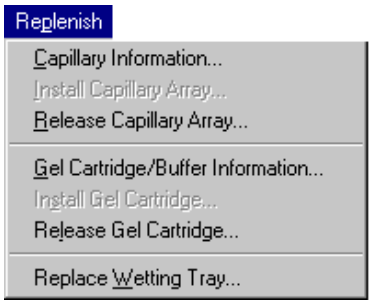
Table 16: Run Module, Log Options Menu



ceqw113e

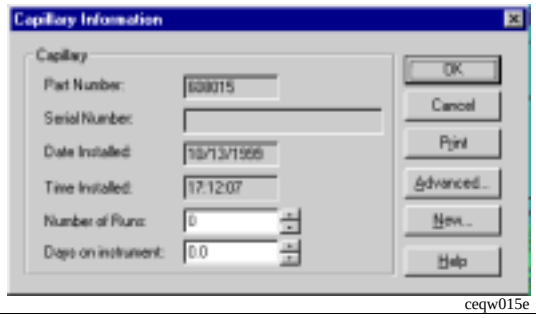
Item	Description
Errors Only	Causes the program to list *errors only* in the Log Window.
All Details	Causes the program to display *all details* in the Log Window listing including all messages and system activity.
Sample Plate Only	Causes the program to only list items related to a running *sample plate* in the Log Window.
Freeze Log	Used to freeze the display in the Log Window. (Freezing the log data does not stop the collection of data, just the display of data.)

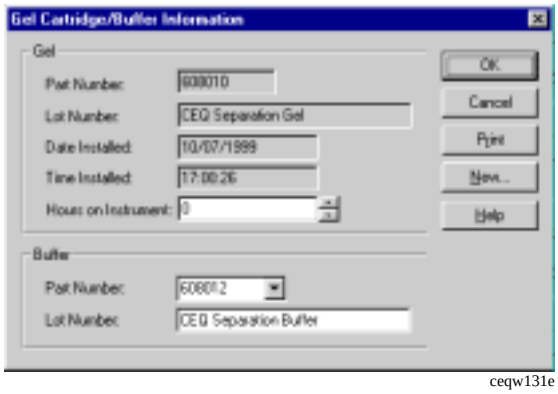
Replenish Menu

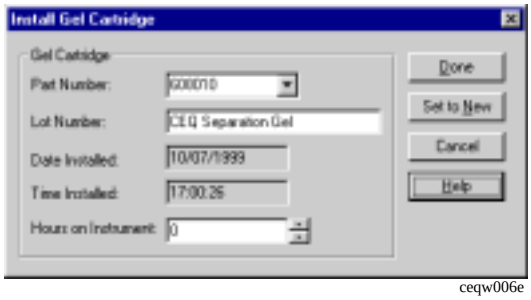
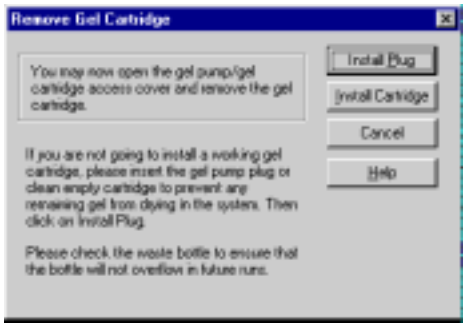
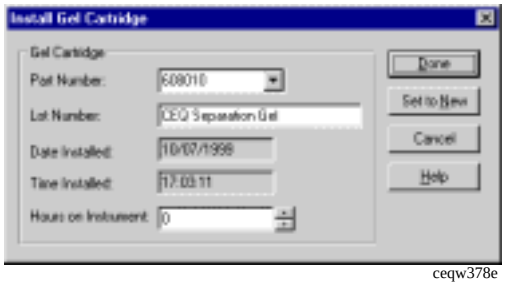


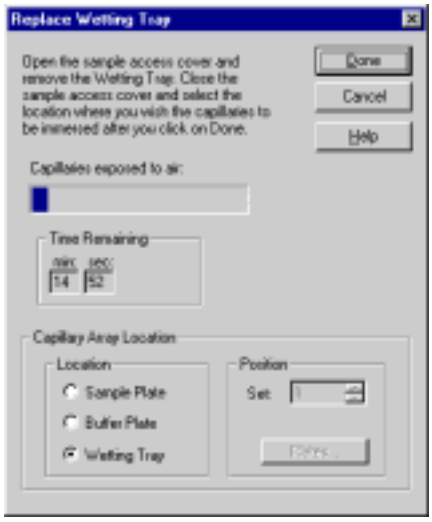
ceqw014e

Table 17: Run Module, Replenish Menu

Item	Description
Capillary Information	Displays the **Capillary Information** dialog box to view the part number and serial number for the capillary array, the number of previous runs and the number of days that the capillary array has been on the instrument. The **Advanced** button shows additional capillary parameters. The **New** button is used to define new capillary array features, such as part number, length and internal diameter. These features differ between the sequence analysis capillary array and the fragment analysis capillary array.  ceqw015e
Install Capillary Array	Used to install the capillary array. Verify or change the information requested, and then install the array.  ceqw117e

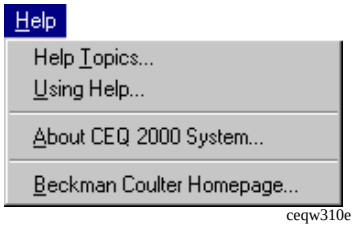
Item	Description
Release Capillary Array	Used to remove the current capillary array. To install a new capillary array, select the **Replace capillary array** radio button. To install a manifold plug, select **Install manifold plug**. To clean the capillary windows, select **Clean capillaries**.  ceqw116e
Gel/Buffer Information	Used to view and/or edit the part number, lot number for the gel and/or buffer, the date and time of gel cartridge installation and the number hours the cartridge has been on the instrument. The **New** button is used to enter part numbers for new gel cartridges or buffers.  ceqw131e

Item	Description
Install Gel Cartridge	Used to install a gel cartridge.  ceqw006e
Release Gel Cartridge	Used to release the gel cartridge in preparation for installing a new cartridge or preparing the system for short or long-term storage. Enter the requested information and then: • Select **Not Installed** and then install the Manifold Plug or a clean, empty cartridge ***OR*** • Select **Install Cartridge** to install a new cartridge.   ceqw378e ceqw379e

Item	Description
Replace Wetting Tray	Used to replace or re-fill the wetting tray.  ceqw392e

Help Menu

Table 18: Run Module, Help Menu



ceqw310e

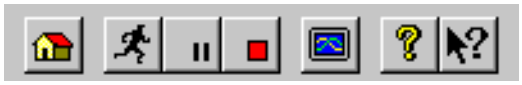
Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ 2000 System	Used to access software, instrument and system information.
Beckman Coulter Homepage	Used to access the Beckman Coulter Homepage on the Internet.

Toolbar Icons

There are six toolbars (Figure 24) used in the Run module: (1) Standard, (2) Direct Control, (3) Data Monitor, (4) Log, (5) Sequence Dye Colors, and (6) Fragment Dye Colors. Each icon of the various toolbars corresponds to a commonly used menu item. The following tables describe the function of each of the toolbar icons.

Figure 24: Run Module, Toolbars

Standard Toolbar



(Table 19)

Direct Control Toolbar

(Table 20)

Data Monitor Toolbar

(Table 21)

Log Toolbar

(Table 22)

Sequence Dye Colors Toolbar

(Table 23)

Fragment Dye Colors Toolbar

(Table 24)

ceqw062e
ceqw070e
ceqw083e
ceqw127e
ceqw922e
cewrf1015

Standard Toolbar

Figure 25: Run Module, Standard Toolbar

ceqw062e

Table 19: Run Module, Standard Toolbar

Button	Description
	***Restore Data Monitor Defaults*** - Used to restore the display configuration of the display monitor to the original default shipped with the instrument.
	***Run Sample Plate*** - Used to open and execute a specific sample plate.
	***Pause*** - Used to pause a running sample plate.

Button	Description
	Stop - Used to stop a running sample plate or direct control process.
	View Last Analysis - Used to execute the Analysis module (Sequence or Fragment) and display the last sample analyzed.
	Help Index - Used to select and print specific topics in the CEQ Help file and search for information by: topic, index entry and/or keyword.
	Context-Sensitive Help - Used to open the Help file related to a specific menu option.

Direct Control Toolbar

Figure 26: Run Module, Direct Control Toolbar

ceqw070e

Table 20: Run Module, Direct Control Toolbarn

	Inject - Used to initiate the injection of the sample prior to separation.
	Separate - Used to initiate the separation of injected samples.
	Fill Capillary with Gel - Used to fill all capillaries with fresh gel. (Any gel present in the capillary is forced out to the specified waste position in the buffer plate or wetting tray.)
	Capillary Temperature - Used to change the capillary holding temperature.
	Denature - Used to initiate the denaturing of samples.
	Optical Alignment - Used to align the lasers with the detection windows of the eight capillaries. Data can be saved and viewed in the Sequence and Fragment Analysis modules.
	Plate Position - Used to select the plate or tray position where the capillaries will be immersed.
	Displays the **Unload Plates** dialog box which is used to: • select the position for capillary immersion in the plate or wetting tray. • move the plates to the Load position at the front of the instrument to load or unload plates.
	Release Gel Cartridge - Used to release the gel cartridge in preparation for installing a new cartridge or preparing the system for short or long-term storage.
	Install Gel Cartridge - Used to install a gel cartridge.

	***Release Capillary Array*** - Used to remove the current capillary array. To install a new capillary array, select the **Replace capillary array** radio button. To install a manifold plug, select **Install manifold plug**. To clean the capillary windows, select **Clean capillaries**.
	***Install Capillary Array*** - Used to install the capillary array. Verify or change the information requested, and then install the array.
	***Replace Wetting Station*** - Used to replace or re-fill the wetting tray.

Data Monitor Toolbar

Figure 27: Run Module, Data Monitor Toolbar

ceqw083e

Table 21: Run Module, Data Monitor Toolbar

	***Unzoom*** - Used to undo one zoom level.
	***Unzoom All*** - Used to undo all zoom levels.
	***Autoscroll On/Off***- Used to scale all data to the last 10 minutes of the run on the X axis and confine the display of data to the pane on the Y axis.
	***Autoscale On/Off*** - Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this icon to turn autoscaling on or off.
	***Pause Data Display*** - Used to pause the display of data. Pausing data does not stop the stream of data, just the display of data.
	***Monitor Baseline*** - Baseline monitoring displays the baseline data trace. To ensure that the optics are working correctly and that the capillaries are clean, view the baseline when a new capillary array is installed and after the capillaries have been filled with gel for the first time. The baseline trace should be low and relatively flat. If **Autosave** is selected from the dialog box, baseline data will be stored under the Sample Data node (for the selected project) in the Data Manager. If Monitor Baseline is not ended manually, baseline data will be collected until a sample plate is run or until six hours have elapsed from the start of the baseline monitoring process.
	***Display Options*** - Invokes the Display Options dialog box used to modify display parameters.

Log Toolbar

Figure 28: Run Module, Log Toolbar

ceqw127e

Table 22: Run Module, Log Toolbar

	***Print*** - Used to print a single pane selected in the Display Area.
	***Print Preview*** - Used to display a facsimile of a hardcopy printout of the active sample plate.

Sequence Dye Colors Toolbar

Figure 29: Run Module, Sequence Dye Colors Toolbar

ceqw922e

Table 23: Run Module, Sequence Dye Colors Toolbar

Icon	Description
A: (red)	The color red is the default color assigned to the Adenine (**A:**) nucleotide bases in the Analyzed Data pane.
C: (black)	Black is the default indicator assigned to the Cytosine (**C:**) nucleotide bases in the Analyzed Data pane.
G: (green)	The color green is the default color assigned to the Guanine (**G:**) nucleotide bases in the Analyzed Data pane.
T: (blue)	The color blue is the default color assigned to the Thymine (**T:**) nucleotide bases in the Analyzed Data pane.

Fragment Dye Colors Toolbar

Figure 30: Run Module, Fragment Dye Colors Toolbar

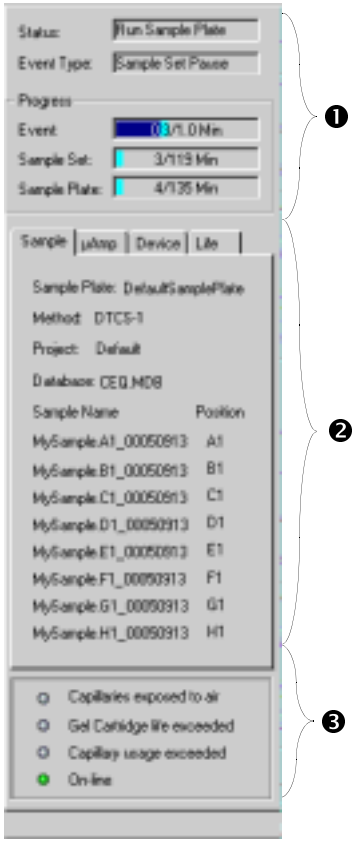
Table 24: Run Module, Fragment Dye Colors Toolbar

Icon	Description
D1 (red)	The color red is the default color assigned to Dye #1 in the Fragment Data pane, and **D1** is the default name.
D2 (black)	The color black is the default color assigned to Dye #2 in the Fragment Data pane, and **D2** is the default name.
D3 (green)	The color green is the default color assigned to Dye #3 in the Fragment Data pane, and **D3** is the default name.
D4 (blue)	The color blue is the default color assigned to Dye #4 in the Fragment Data pane, and **D4** is the default name.

Status Monitor

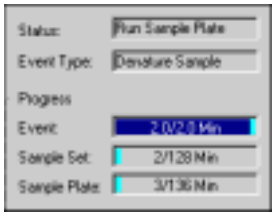
The Status Monitor displays the state of the current run. Figure 31 shows the status monitor and Table 25 describes the areas.

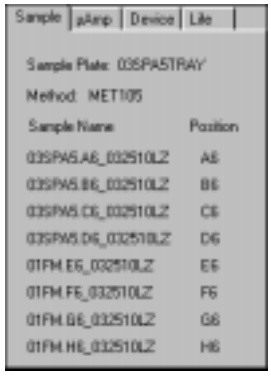
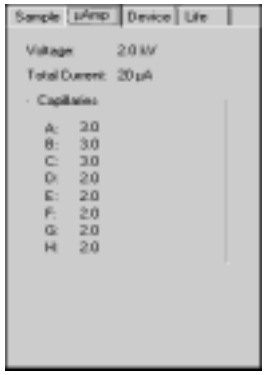
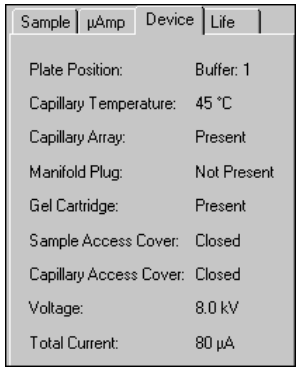
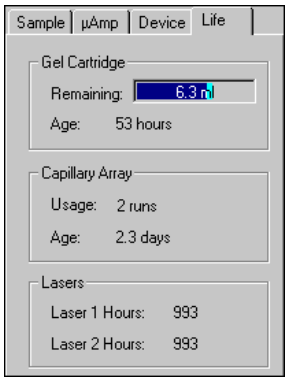
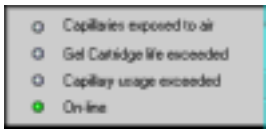
Figure 31: Run Module, Status Monitor Displays



ceqw091e

Table 25: Run Module, Status Monitor Displays

Item	Function
❶	***Progress Indicator*** - This area of the Status Monitor shows the Status, Event Type and Progress of a running sample plate or process.  ***Times displayed are approximations.***  ceqw210e

Item	Function
❷	***Information Tabs*** - Toggles between the tabs shown below. • ***Sample*** Lists the sample plate, sample names and the method being used.  ceqw255e • ***µAmp*** Shows the approximate overall microamperes (µA) and voltage used during the separation.  ceqw210e • ***Device*** Displays the condition or value of devices or parameters.  ceqw211e • ***Life*** Displays the remaining gel, capillary usage and laser hours.  ceqw212e
❸	***Indicators*** - Provides indicators that pertain to capillary usage, gel cartridge life and on-line status. When the life of the gel or capillary array is exceeded, the associated indicator turns red. When the system is on-line, the indicator is green.  ceqw327e

Window Selection Tabs

The Window Selection tabs provide access to the following windows: Data Monitor, Direct Control, Log and Instrument Data. These windows are described on the following pages.

Data Monitor Window

The Data Monitor window is shown in Figure 32. This window is accessed from the Run window by selecting the Data Monitor *tab.* The window displays information associated with a sample's analysis.

Figure 32: Run Module, Data Monitor Window

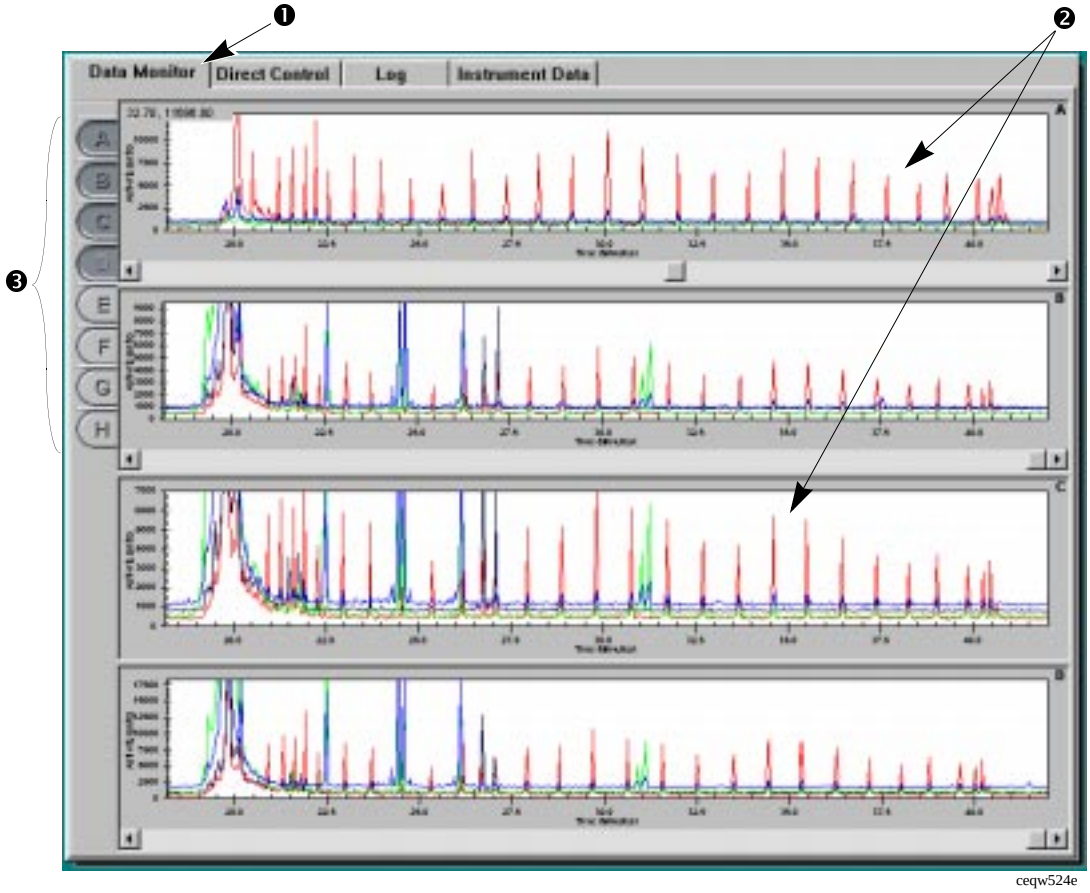


Table 26: Run Module, Data Monitor Window Descriptions

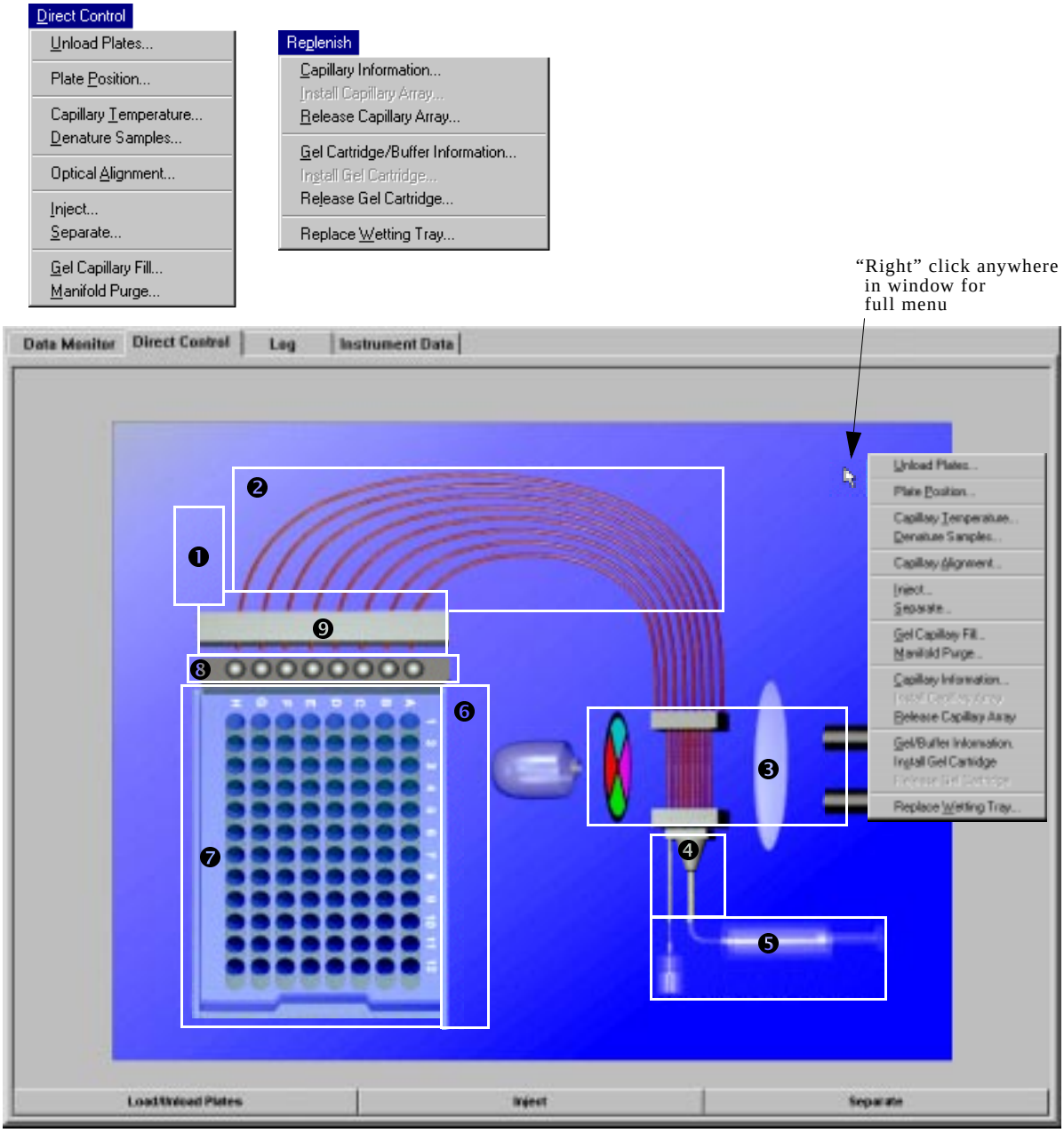
Item	Description
❶	***Window Selection Tab*** - Select this tab to access the Data Monitor window.
❷	***Data Monitor Window*** - This window displays the data for the running sample plate for the selected capillary buttons.
❸	***Capillary Buttons*** - These buttons (**A** through **H**) represent the eight capillaries of the array.

Direct Control Window

The Direct Control window is shown in Figure 33. This window is accessed from the Run window by selecting the Direct Control *tab.*

This window contains hot areas and function buttons that perform some of the distinct tasks listed in the “Direct Control” and “Replenish” drop-down menus.

Figure 33: Run Module, Direct Control Window



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The circled numbers shown on the window in Figure 33 represent the locations of the hot areas in the Direct Control window. Each hot area, when selected, initiates a unique Direct Control task. Table 27 lists the tasks available in this window.

Table 27: Direct Control Window, Hot Areas

Item	Description
❶	***Capillary Temperature*** **-** Used to change the capillary temperature.
❷	***Gel Capillary Fill*** **-** Used to fill all capillaries with fresh gel. (Any gel present in the capillary is forced out to the specified waste position in the buffer plate or wetting tray.)
❸	***Optical Alignment*** - Used to align the lasers with the detection windows of the eight capillaries. Data can be saved and viewed in the Sequence or Fragment Analysis module.
❹	***Manifold Purge*** - Used to clear the manifold of air pockets and/or remaining DNA fragments before the next separation process.
❺	***Install/Release Gel Cartridge*** - Used to release and/or install a gel cartridge.
❻	***Denature Samples*** - Used to initiate the denaturing of samples.
❼	***Plate Position*** - Used to select the plate or tray position where the capillaries will be immersed.
❽	***Wetting Tray*** - Used to replace or re-fill the wetting tray.
❾	***Install/Release Capillary*** - Used to install the capillary array. Verify or change the information requested, and then install the array.
	Load/Unload Plates - Displays the **Unload Plates** dialog box. This dialog box is used to:  ceqw124e • select the position for capillary immersion in the plate or wetting tray. • move the plates to the Load position at the front of the instrument to load or unload plates.
	Inject - Used to initiate the injection of the sample prior to separation.  ceqw125e
	Separate - Used to initiate the separation of injected samples.  ceqw126e

Log Window

The Log window (Figure 34) is accessed from the Run window by selecting the Log *tab.* The log window provides all of the messages and activity for a sample plate run.

Figure 34: Run Module, Log Window

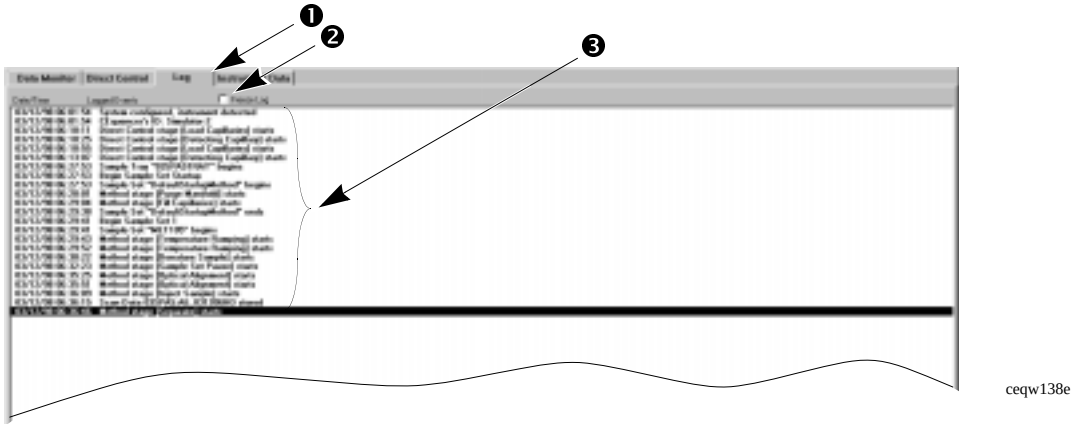


Table 28: Run Module, Log Window Descriptions

Item	Description
❶	***Window Selection Tab*** - Select this tab to access the Log window.
❷	***Freeze Log*** - Used to freeze the display in the Log window. (Freezing the log data does not stop the collection of data, just the display of data.)
❸	The ***Display Area*** provides the list of logged events.

Instrument Data Window

The Instrument Data window (Figure 35) is accessed from the Run window by selecting the Instrument Data *tab*. This window displays the current for the eight capillaries and the voltage level of the instrument for the current run.

Figure 35: Run Module, Instrument Data Window

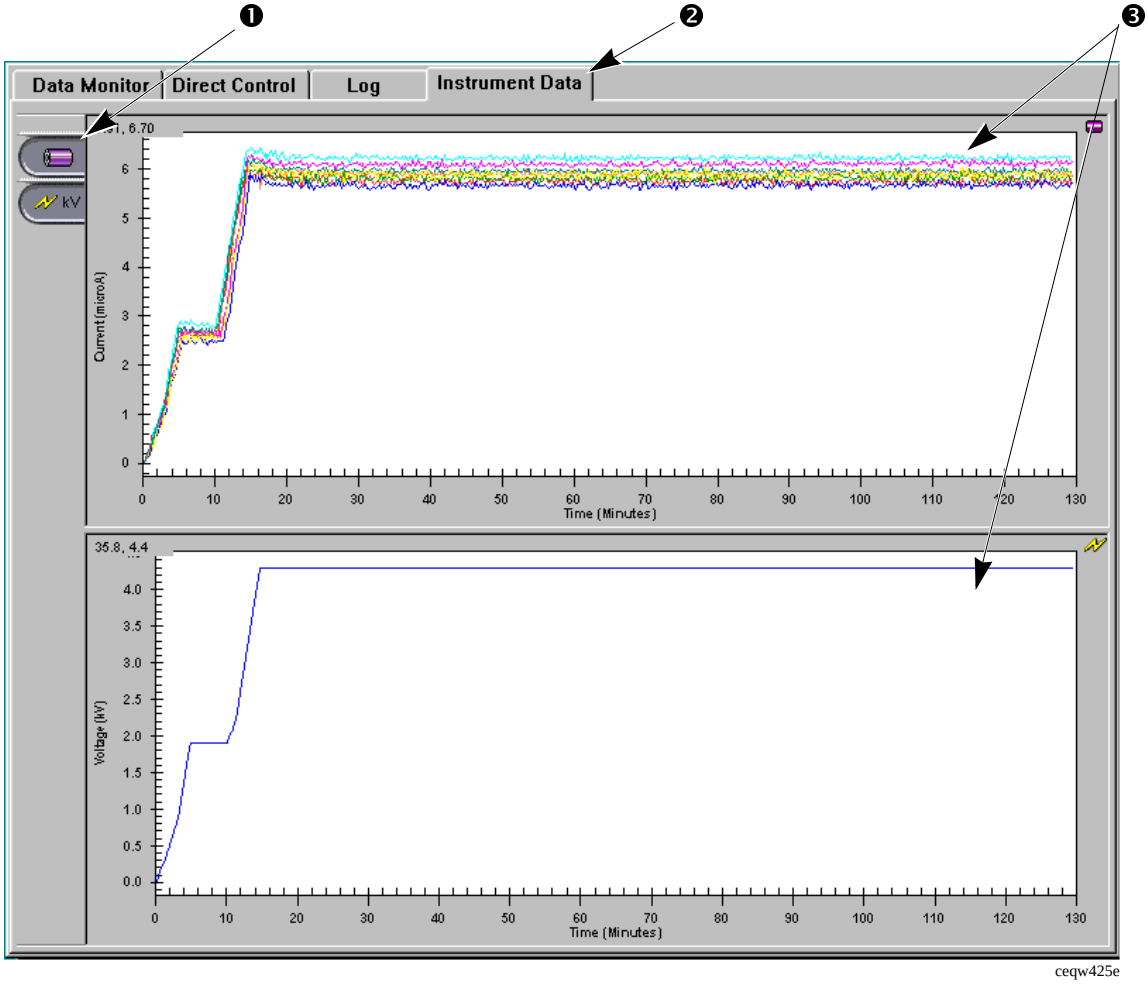


Table 29: Run Module, Log Window Descriptions

Item	Description
❶	***Current and Voltage Buttons*** - Used to select panes for display.
❷	***Window Selection Tab*** - Select this tab to access this window.
❸	The ***Display Area*** displays the current and voltage levels of selected current run.

Sequence Analysis Module

The Sequence Analysis module is used to view, analyze, compare, manipulate and print data of the following types:

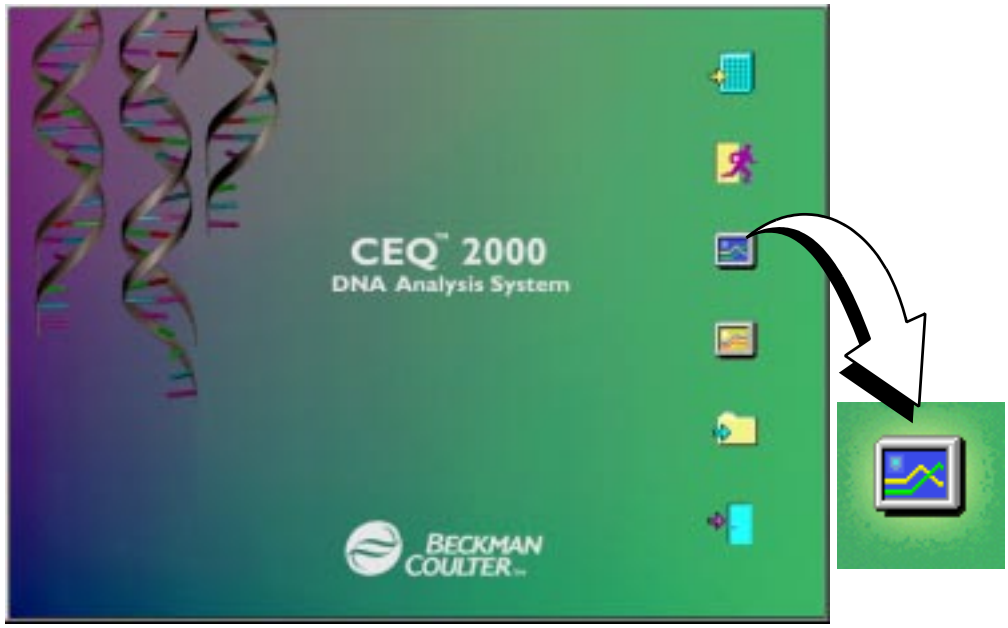
- Raw Data*
- Current Data*
- Voltage Data*
- Analyzed Data
- Base Sequences
- Optical Scan Data*
- Baseline Data*
- Quality Parameters*

* These types of data cannot be manipulated.

This module accepts raw data and analyzed data. Editing and re-analysis functions provide the capability to verify the accuracy of the base calls. These data can also be exported to third party packages for further analysis.

Selecting the Sequence Analysis *icon* from the Main Menu (Figure 36) executes this module.

Figure 36: Main Menu, Sequence Analysis Icon



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Main Window

The main window of the Sequence Analysis module is shown in Figure 37 and is described in Table 30.

Figure 37: Sequence Analysis Module, Main Window

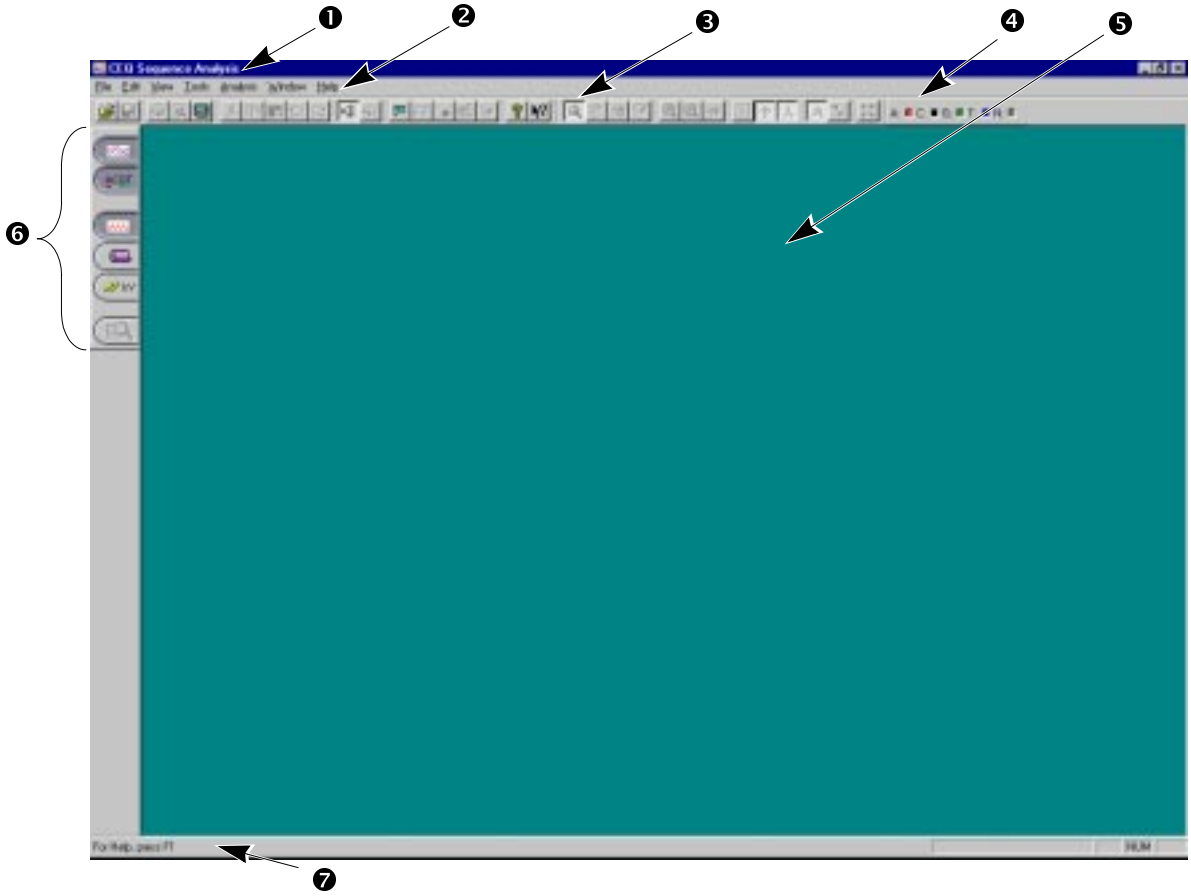

Table 30: Sequence Analysis Module, Main Window Descriptions

Item	Description
❶	The ***Title Bar*** showing the module name (*CEQ Sequence Analysis*).
❷	The ***Menu Bar*** is a list of the menu options (see ***"Menu Bar Options"*** on page 2-41).
❸	The ***Toolbar*** contains the icons that execute pre-defined functions (see ***"Toolbar Icons"*** on page 2-52).
❹	Double-clicking on a base sequence text letter (**A**, **C**, **G**, **T**) in the ***Dye Colors*** box brings up the **Color** dialog box. Change the dye color displayed *for that particular* dye.
❺	This ***Display Area*** graphically displays the opened data.

Item	Description
❻	 ***Analyzed Data*** Displays the data that has been analyzed for the active sample. ***Base Sequence*** Displays a text view of the bases from the analyzed data for the active sample. ***Raw Data*** Displays the raw data for the active sample. ***Current*** Displays the current data for the active sample. ***Voltage*** Displays voltage data for the active sample. ***Compare Data*** Used to compare the active analyzed data set against another analyzed data set.
❼	The ***Status Bar*** displays information concerning the current selection.

Menu Bar Options

The menu bar has the options shown in Figure 38. Tables 31 through 37 provide descriptions of the menu options.

Figure 38: Sequence Analysis Module, Menu Bar Options

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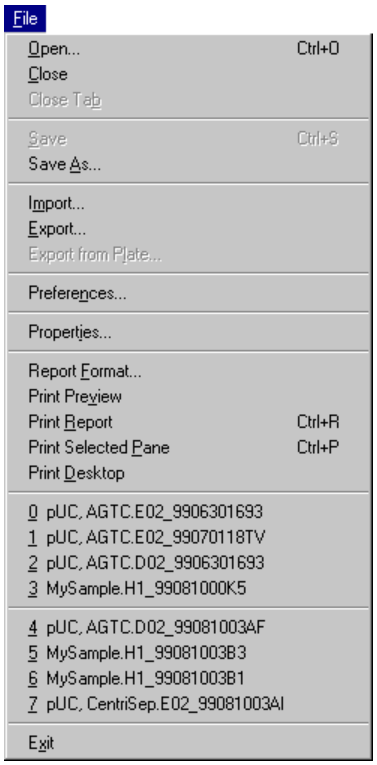
File Menu

Table 31: Sequence Analysis Module, File Menu



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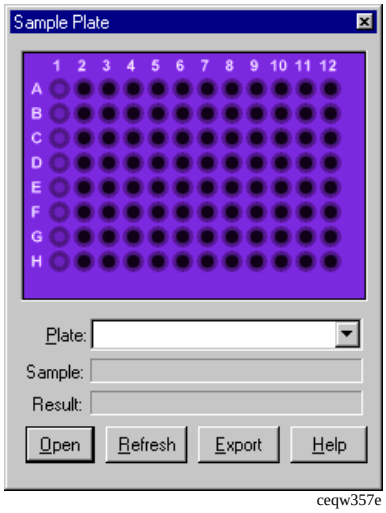
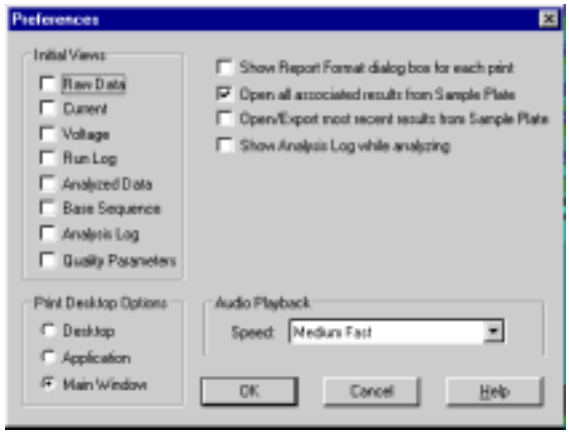
Item	Description
Open	Used to open: Sample Data, Sequence Analysis Parameters, Optical Scan Data, Sequence Results and Sample Plate Results.
Close	Closes the open sample.
Close Tab	Closes the active tab.
Save	Saves existing data.
Save As	Used to save data to a new name.
Import	Used to locate data to be imported. The **Standard Chromatogram Format** (***.scf**) is the only available format for importing into this module.
Export	Used to export data in one of the following formats: • Standard Chromatogram Format Ver 3.00 (*.scf) • Standard Chromatogram Format Ver 2.10 (*.scf) • Tab Delimited ASCII Text (*.txt) • FASTA and Quality (*.fasta) • PHRED and SCF (*.phd.1) • SEQ Sequence Text(*.seq)

Item	Description
Export from Plate	Used to open or export samples from a specific sample plate. Multiple samples can be selected using the **Shift** key. (The **Sample Plate** toolbar must be open to use this menu option.)  ceqw357e
Preferences	Used to: • view or change what panes will be displayed when the Sequence Analysis module is first opened. • show the **Report Format** dialog box when **Print Report** is selected from the **File** menu. • open all associated results that belong to a sample when a sample(s) is opened. • open/export the most recent results from a sample plate. • show the Analysis Log during analysis. • print the entire desktop. • print the application window, including all toolbars. • print only the displayed panes. • select audio playback speed.  ceqw432e

Item	Description
Properties	Displays the current database, modification date as well as providing other information.
Report Format	Used to specify the format of the report that will be generated for the active sample.
Print Preview	Used to display a facsimile of a hardcopy printout of the selected sample plate.
Print Report	Used to print the report.
Print Selected Pane	Used to print the selected pane.
Print Desktop	Used to print the desktop, application or main window as defined in the **Preferences** dialog box.
Recent Objects	Lists the most recently opened objects. The first set of four is sample data and the second set of four is sequence results.
Exit	Closes the Sequence Analysis module.

Edit Menu

Table 32: Sequence Analysis Module, Edit Menu

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Item	Description
Undo	Used to undo the last action performed.
Redo	Used to redo the last undo action.
Cut	Used to cut selected bases.
Copy	Used to duplicate selected bases.
Paste	Used to insert one or more copied or cut bases.
Audio Enable	Toggles between enabling or disabling audio. When enabled, as a base letter is typed, in **Edit** mode, the letter is announced audibly.
Audio Playback	Used to audibly announce a series of selected base sequence text.

View Menu

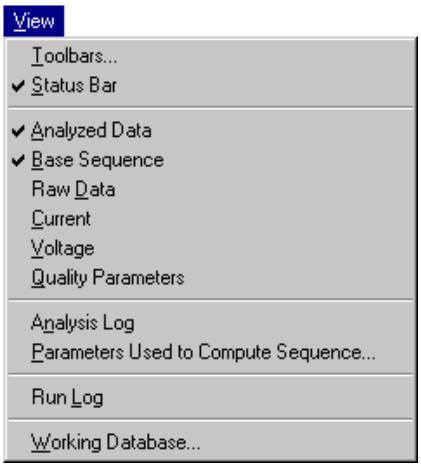

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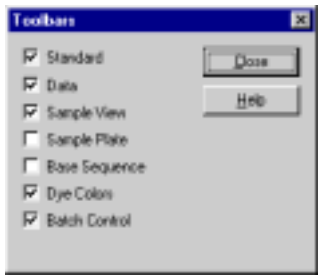
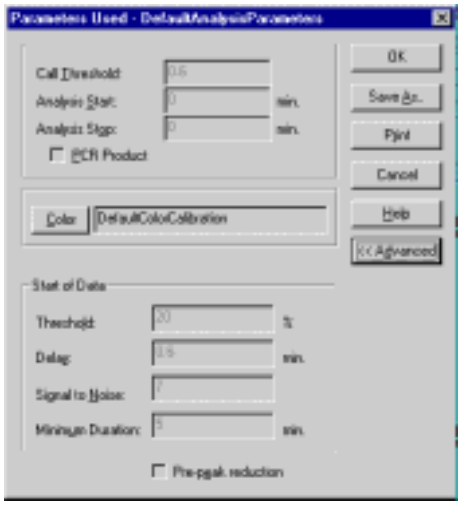
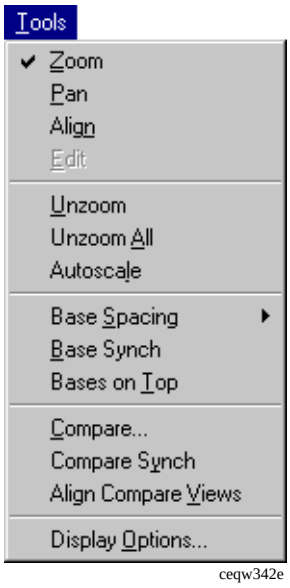
Table 33: Sequence Analysis Module, View Menu

Item	Description
Toolbars	Used to select which toolbars to display.  ceqw360e
Status Bar	Toggles between displaying or not displaying the Status Bar.
Analyzed Data	Displays Analyzed Data.
Base Sequence	Displays Base Sequence Data.
Raw Data	Displays Raw Data.
Current	Displays Current Data.
Voltage	Displays Voltage Data.
Quality Parameters	Displays Quality Parameters.
Analysis Log	Displays the Analysis Log.

Item	Description
Parameters Used to Compute Sequence	Used to: • View parameters used to perform the base-calling operation on the active sample. • Save the values as a new parameter set. • View the color calibrations for the sequence analysis parameters. (These values show the cross-talk between the filters and the emissions for A, C, G, and T.) • View the computed color calibrations after the run. • View the color calibration values prior to the run. • Add a successful color calibration to the list of available color calibrations. If **Advanced** is selected, the following additional information used to detect the start of data can also be viewed. • The % Threshold, • the Delay, • the Signal to Noise ratio, and • the Minimum Duration.  ceqw930e
Run Log	Displays the messages received from the instrument during the sample run.
Working Database	Displays the name of the database in use.

Tools Menu



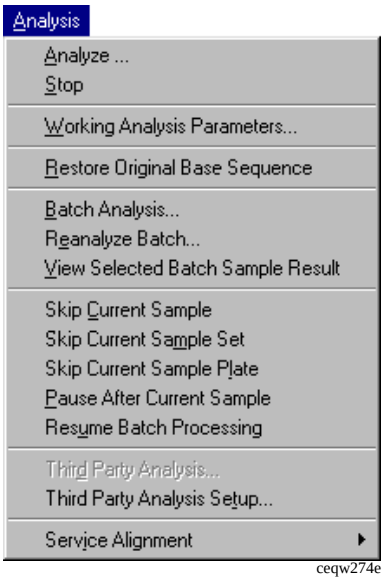
ceqw342e

Table 34: Sequence Analysis Module, Tools Menu

Item	Description
Zoom	Used to magnify a specific area of data in the display.
Pan	Used to move analyzed data in the X and Y direction.
Align	Used to visually align bases and peaks in the analyzed data pane.
Edit	Used to insert, change and/or delete bases.
Unzoom	Used to undo one zoom level.
Unzoom All	Used to undo all zoom levels.
Autoscale	Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this item to turn autoscaling on or off.
Base Spacing	Used to set the space between base sequence text in groups of 0, 3, 5 or 10.
Base Synch	With both the Analyzed Data and Base Sequence panes open, synchronizes the analyzed data display with the highlighted base sequence text, showing the corresponding peak or peaks between two hairlines for the selected base.
Bases on Top	Toggles the base sequence text between displaying on the top or bottom in the analyzed data pane.
Compare	Used to select an analyzed data set to compare to the current analyzed data set.
Compare Synch	Used to synchronize scaling, zooming and panning of two analyzed data sets while in the **Compare** mode.
Align Compare Views	Used to align selected points of each of the displayed analyzed data sets while in the **Compare** mode.

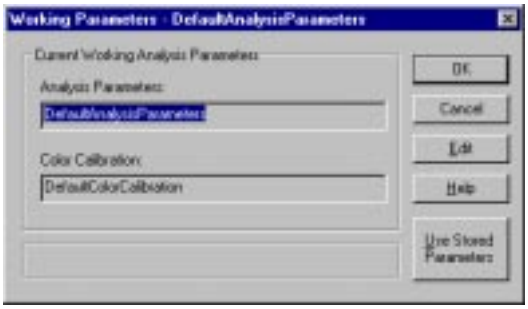
Item	Description
Display Options	Invokes the **Display Options** dialog box used to modify any or all of the following parameters: • Title • X Axis Options • Y Axis Options • Dye Traces • Current Traces • Colors • Quality Parameters See immediate changes in the selected pane by clicking on **Apply**.  ceqw098e ***Double-clicking the left mouse button in the selected pane, also brings up this dialog box.***

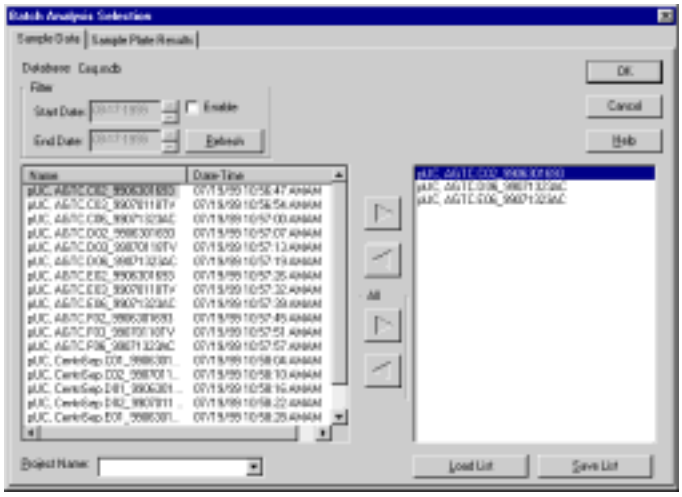
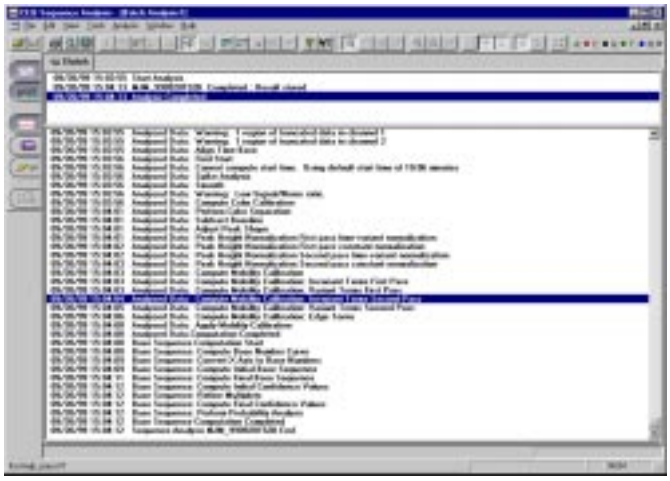
Analysis Menu



ceqw274e

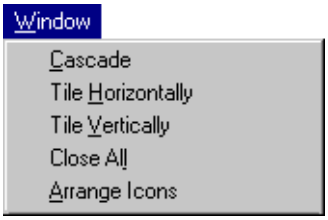
Table 35: Sequence Analysis Module, Analysis Menu

Item	Description
Analyze	Used to: • view the current working analysis parameters. (The analysis parameter set defines the threshold above which data will be considered peaks, the threshold below which bases will be called as 'N's, and four parameters used to detect the start of valid data [the start and end times for the analysis of raw data, the delay, the signal to noise ratio and the minimum duration]). • view and/or change the color calibration name and values. These values show the cross-talk between the filters and the emissions for A, C, G, and T. • view and/or change the working analysis parameters (maintained in memory for the duration of the application session). • begin the analysis when the **OK** button is selected.  ceqw362e
Stop	Terminates the executing analysis.
Working Analysis Parameters	Used to view and/or change the analysis parameters to be used for subsequent analyses.
Restore Original Base Sequence	Used to return analyzed data display and base sequence text back to its original state (losing all edits).

Item	Description
Batch Analysis	Used to analyze multiple Sample Data or Sample Plate Results sequentially. Invokes the following dialog box.  ceqw386e Once files are transferred to the right-side list box and **OK** is selected, batch processing begins and a window, similar to the one below, is displayed.  ceqw388e
Reanalyze Batch	After performing a batch analysis, use this option to perform the analysis again.
View Selected Batch Sample Result	After performing a batch analysis, use this item to view the results of a selected sample in the upper pane of the **Batch Analysis** window.

Item	Description
Skip Current Sample	While performing a batch analysis, use this item to pass over the *sample* currently being analyzed. (This item is not available if **Sample Plate Results** was selected from the **Batch Analysis Selection** dialog box.)
Skip Current Sample Set	While performing a batch analysis, use this item to pass over the *sample set* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
Skip Current Sample Plate	While performing a batch analysis, use this item to pass over the sample *plate* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
Pause After Current Sample	Used to momentarily stop a batch analysis after the currently running sample has been analyzed. Use **Resume Batch Processing** to continue the analysis.
Resume Batch Processing	Used to resume a batch analysis after it has been paused.
Third Party Analysis	Used to launch the third party analysis package specified in the **Third Party Analysis Setup** dialog box.
Third Party Analysis Setup	Used to specify the path, third party analysis package (loaded on your PC) and the export format of the data.

Window Menu

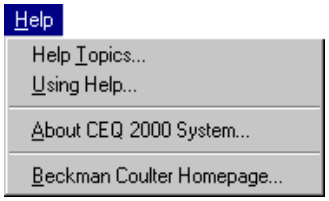


ceqw343e

Table 36: Sequence Analysis Module, Window Menu

Item	Description
Cascade	Cascades the open windows.
Tile Horizontally	Tiles the windows in a horizontal orientation.
Tile Vertically	Tiles the windows in a vertical orientation.
Close All	Closes any currently active window(s).
Arrange Icons	Automatically arranges the icons.

Help Menu



ceqw310e

Table 37: Sequence Analysis Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ 2000 System	Used to access software, instrument and system information.
Beckman Coulter Homepage	Used to access the Beckman Coulter Homepage on the Internet.

Toolbar Icons

There are seven toolbars (Figure 39) used in the Sequence Analysis module: (1) Standard, (2) Data, (3) Sample View, (4) Sequence Dye Colors, (5) Batch Control, (6) Sample Plate and (7) Base Sequence. Each icon of these toolbars corresponds to a commonly used menu item. By default, all toolbars (except Sample Plate and Base Sequence) are displayed when the Sequence Analysis module is opened. The tables below describe the functions of each of the toolbar icons.

Figure 39: Sequence Analysis Module Toolbars

Standard Toolbar

(Table 38)

Data Toolbar

(Table 39)

Sample View Toolbar

(Table 40)

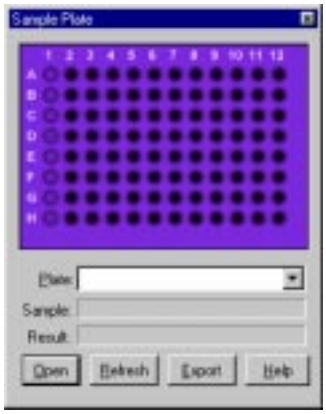
Sequence Dye Colors Toolbar

(Table 41)

Batch Control Toolbar

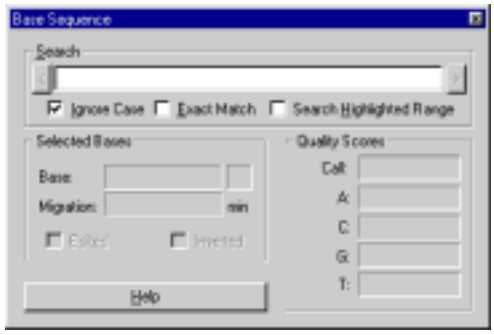
(Table 42)

Sample Plate Toolbar



(Figure 45)

Base Sequence Toolbar



(Figure 46)

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ceqw329e
ceqw331e
ceqw394e
ceqw393e
ceqw434e
ceqw435e

Standard Toolbar

Figure 40: Sequence Analysis Module, Standard Toolbar


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Table 38: Sequence Analysis Module, Standard Toolbar

Icon	Description
	Open - Used to open: Sample Data, Sequence Analysis Parameters, Optical Scan Data, Sequence Results and Sample Plate Results.
	Save - Saves existing data.
	Print - Used to print the report.
	Print Preview - Used to display a facsimile of a hardcopy printout of the selected sample plate.
	Print Desktop - Used to print the desktop, application or main window as defined in the **Preferences** dialog box.
	Cut - Used to cut selected bases.
	Copy -Used to duplicate selected bases.
	Paste - Used to insert one or more copied or cut selected bases.
	Undo - Used to undo the last action performed.
	Redo - Used to redo the last undo action.
	Audio Enable - Toggles between enabling or disabling audio. When enabled, as a base letter is typed, in **Edit** mode, the letter is announced audibly.
	Audio Playback - Used to audibly announce a series of selected base sequence text.
	Working Analysis Parameters - Used to view and/or change the analysis parameters to be used for subsequent analyses.
	Analyze - Used to: • view the current working analysis parameters. (The analysis parameter set defines the threshold above which data will be considered peaks, the threshold below which bases will be called as 'N's, and four parameters used to detect the start of data to be analyzed [the start and end times for the analysis of raw data, the delay, the signal to noise ratio and the minimum duration.]) • view and/or change the color calibration name and values. These values show the cross-talk between the filters and the emissions for A, C, G, and T. • view and/or change the working analysis parameters (maintained in memory for the duration of the application session). • begin the analysis when the **OK** button is selected.

Icon	Description
	Stop - Terminates the executing analysis.
	Restore Original Base Sequence - Used to return analyzed data display and base sequence text back to its original state (losing all edits).
	Third Party Analysis - Used to launch the third party analysis package specified in the **Third Party Analysis Setup** dialog box.
	Help Index - Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
	Context-Sensitive Help - Used to open the Help file related to a specific menu option.

Data Toolbar

Figure 41: Sequence Analysis Module, Data Toolbar

ceqw329e

Table 39: Sequence Analysis Module, Data Toolbar

Icon	Description
	Zoom Mode - Used to magnify a specific area of data in the display.
	Pan Mode - Used to move analyzed data in the X and Y direction.
	Align Mode - Used to visually align bases and peaks in the analyzed data pane.
	Edit Mode - Used to insert, change and/or delete bases.
	Unzoom - Used to undo one zoom level.
	Unzoom All - Used to undo all zoom levels.
	Autoscale - Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this item to turn autoscaling on or off.
3	***Base Spacing*** - Used to set the space between base sequence text in groups of 0, 3, 5 or 10.
	Base Synch - With both the Analyzed Data and Base Sequence panes open, synchronizes the Analyzed Data display with the highlighted base sequence text showing the corresponding peak or peaks between two hairlines for the selected base.
	Bases on Top - Toggles the base sequence text between displaying on the top or bottom in the analyzed data pane.
	Compare Synch - Used to synchronize scaling, zooming and panning of two analyzed data sets while in the **Compare** mode.

Icon	Description
	***Align*** - Used to align selected points of each of the displayed analyzed data sets while in the **Compare** mode.
	***Display Options*** -Invokes the **Display Options** dialog box.

Sample View Toolbar

Figure 42: Sequence Analysis Module, Sample View Toolbar

ceqw331e

Table 40: Sequence Analysis Module, Sample View Toolbar

Icon	Description
	***Analyzed Data*** - Displays the data that has been analyzed for the active sample.
	***Base Sequence*** - Displays a text view of the bases from the analyzed data for the active sample.
	***Raw Data*** - Displays the raw data for the active sample.
	***Current*** - Displays the current data from the capillary arrays for the active sample.

Icon	Description
	***Voltage*** - Displays the voltage data from the instrument for the active sample.
	***Compare Data*** - Used to compare the active analyzed data set against another analyzed data set.

Dye Colors Toolbar

Figure 43: Sequence Analysis Module, Dye Colors Toolbar

ceqw394e

Table 41: Sequence Analysis Module, Dye Colors Toolbar

Icon	Description
A: (red)	The color red is the default color assigned to the Adenine (**A:**) nucleotide bases in the Analyzed Data pane.
C: (black)	Black is the default indicator assigned to the Cytosine (**C:**) nucleotide bases in the Analyzed Data pane.
G: (green)	The color green is the default color assigned to the Guanine (**G:**) nucleotide bases in the Analyzed Data pane.
T: (blue)	The color blue is the default color assigned to the Thymine (**T:**) nucleotide bases in the Analyzed Data pane.
N: (gray)	The color gray is the default color assigned to ambiguous nucleotide bases in the Analyzed Data pane.

Batch Control Toolbar

Figure 44: Sequence Analysis Module, Batch Control Toolbar

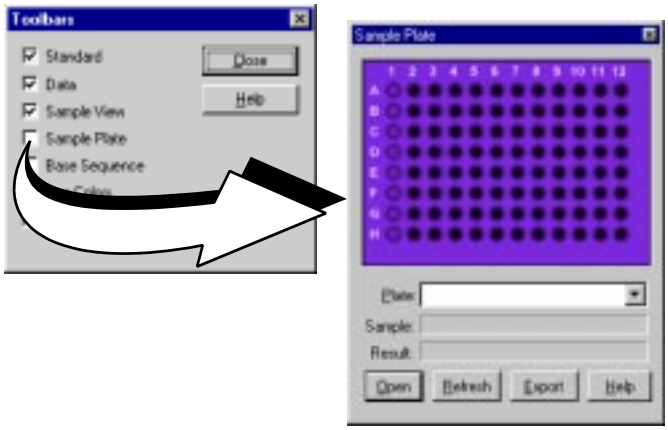
ceqw393e

Table 42: Sequence Analysis Module, Batch Control Toolbar

Icon	Description
	***Batch Analysis*** - Used to analyze multiple Sample Data or Sample Plate Results sequentially.
	***Reanalyze Batch*** - After performing a batch analysis, use this option to perform the analysis again.
	***Stop Analysis*** - Terminates the executing analysis.
	***Pause*** - Used to momentarily stop a batch analysis after the currently running sample has been analyzed. Use **Resume Batch Processing** to continue the analysis.
	***Resume*** - Used to resume a batch analysis after it has been paused.
	***Skip Sample*** - While performing a batch analysis, use this item to pass over the sample currently being analyzed. (This item is not available if **Sample Plate Results** was selected from the **Batch Analysis Selection** dialog box.)
	***Skip Row*** - While performing a batch analysis, use this item to pass over the sample set currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
	***Skip Plate*** - While performing a batch analysis, use this item to pass over the sample plate currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
	***View Results*** - After performing a batch analysis, use this item to view the results of a selected sample in the upper pane of the **Batch Analysis** window.

Sample Plate Toolbar

Figure 45: Sequence Analysis Module, Sample Plate Toolbar



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Accessed from the **View | Toolbars** menu option.

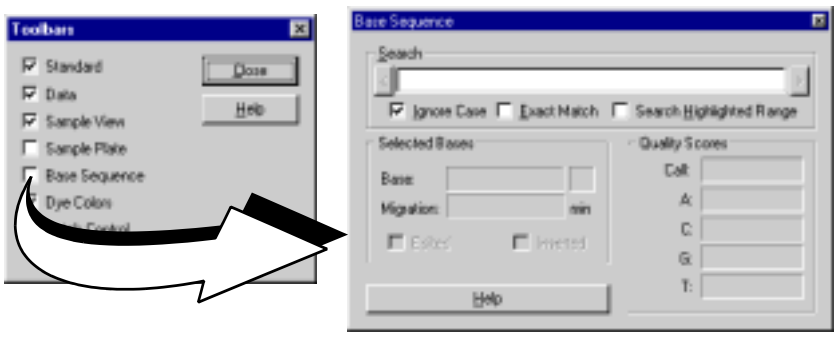
Used to specify the samples to open or export. Select the desired plate from the drop-down menu. Valid samples appear as filled samples. Wells not specified as samples appear dark, or empty. Click on the samples to open or export.

To view the name of a sample and associated result, position the cursor over the sample. The sample and result names are displayed in the **Sample** and **Result** text boxes.

To select contiguous samples, click on the first sample in the series, hold down the* Shift *key and then click on the last sample in the series. The selected samples are highlighted.

Base Sequence Toolbar

Figure 46: Sequence Analysis Module, Base Sequence Toolbar



ceqw435e

Accessed from the **View | Toolbars** menu option.

Used to search for specific base sequences and to view quality parameters for a base or contiguous group of bases.

Search

- Use an asterisk to search for specified characters prior to the asterisk.
- Use ? to search for any character within the string of characters specified.
- Use brackets [ ] to search for any character within the brackets.
- To search for the text regardless of case, check the **Ignore Case** checkbox. To search for the exact match for text (i.e., not IUB codes), check the **Exact Match** checkbox. To search for text within a range, highlight the range of interest in the base sequence pane and check the **Search Highlighted Range** checkbox.
- Use the forward and back arrows to search backwards and forwards through the base sequence text.
- The IUB codes you may enter when the **Exact Match** checkbox is unchecked are listed below.

  N = G, A, T, or C
  V = G, A, or C
  B = G, T, or C
  H = A, T, or C
  D = G, A, or T
  K = G or T
  S = G or C
  W = A or T
  M = A or C
  Y = C or T
  R = A or G

Fragment Analysis Module

The Fragment Analysis module processes fragment data from the CEQ platform and provides size and allele information for detected peaks. The results may be viewed, exported and printed. Parameters used to identify alleles are organized as locus tags that can be customized by the user.

Analysis parameter sets provide parameters specific to an experiment, allowing the user to define size calibration and locus tags to be used for processing data. The analysis of data can be performed manually or automatically. The editing and re-analysis functions provide the capability to verify the accuracy of the peaks.

The Fragment Analysis module is used to graphically view, analyze, manipulate, compare and print data of the following types:

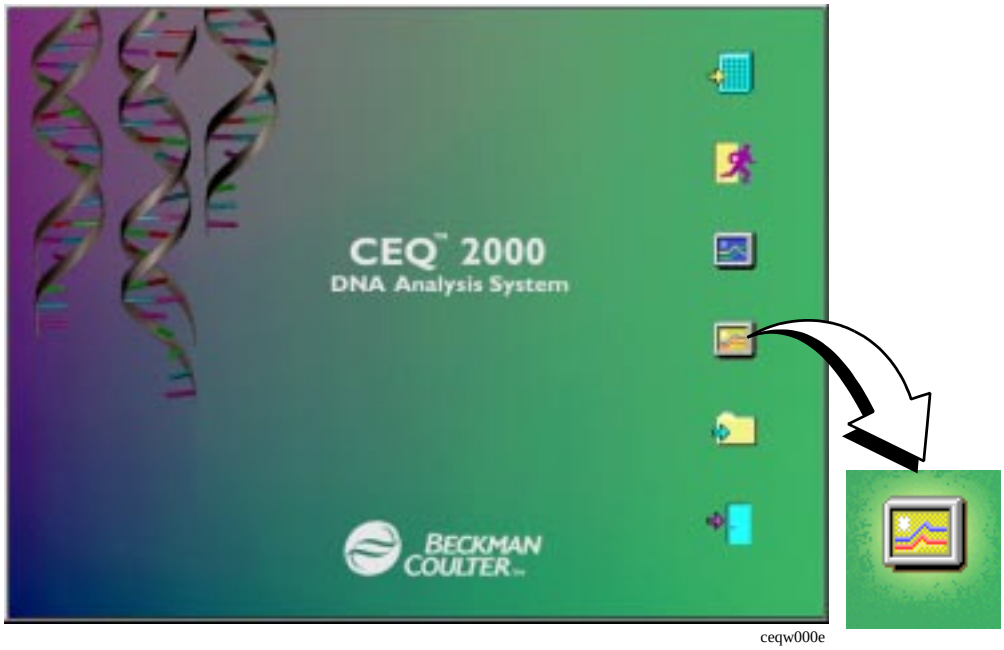
- Raw Data*
- Fragment Data
- Fragment Lists
- Information about locus tags and assigned alleles

Also available are Current Data*, Voltage Data* and Analyzed Data*.

* These types of data cannot be manipulated.

Selecting the Fragment Analysis *icon* from the Main Menu (Figure 47) executes this module.

Figure 47: Main Menu, Fragment Analysis Icon



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Main Window

The main window of the Fragment Analysis module is shown in Figure 48 and described in Table 43.

Figure 48: Fragment Analysis Module, Main Window

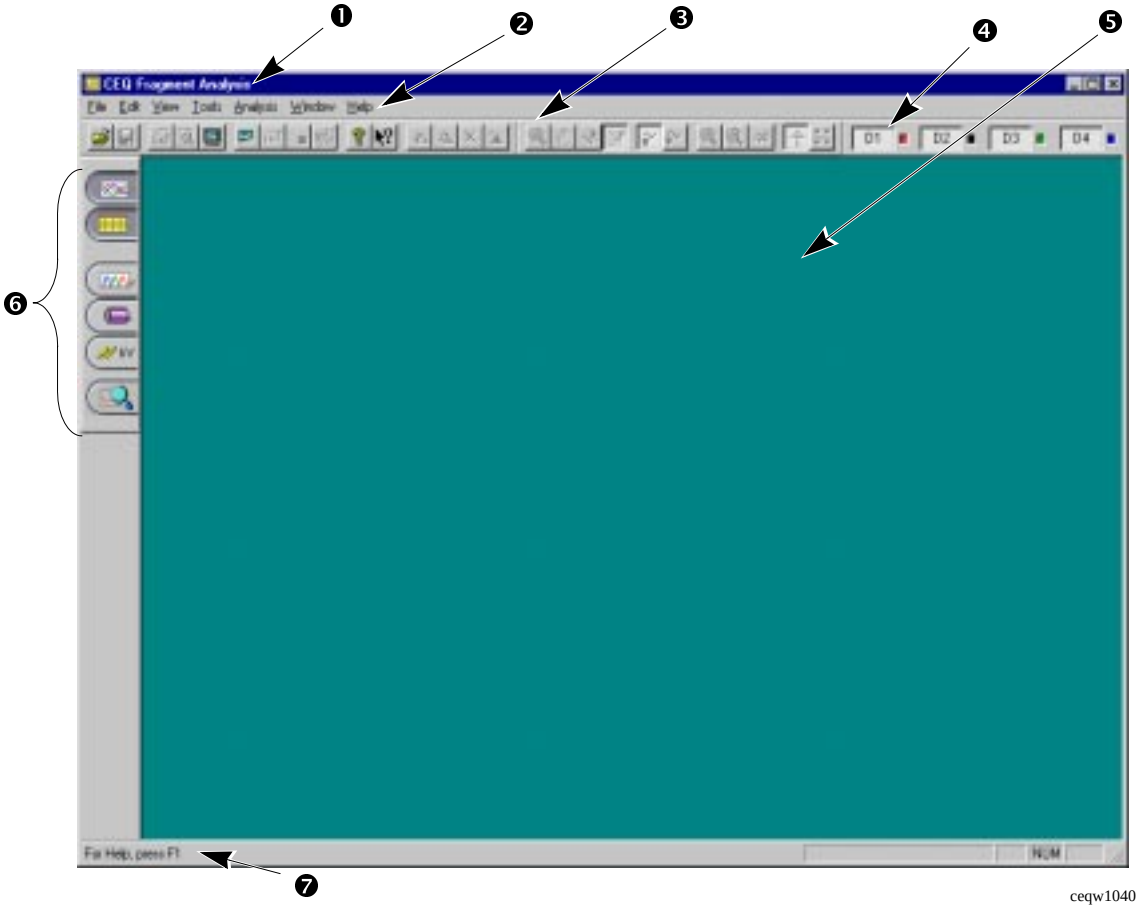
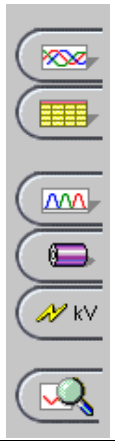


Table 43: Fragment Analysis Module, Main Window Descriptions

Item	Description
❶	The ***Title Bar*** showing the module name (*CEQ Fragment Analysis*).
❷	The ***Menu Bar*** is a list of the menu options (see ***"Menu Bar Options"*** on page 2-62).
❸	The ***Toolbar*** contains the icons that execute pre-defined functions (see ***"Toolbar Icons"*** on page 2-74).
❹	Double-clicking on a dye name (**D1, D2, D3, D4**) in the ***Dye Colors*** box brings up the **Color** dialog box. Change the dye color displayed *for that particular* dye. Note ***To activate this toolbar, select View | Toolbars from the menu and check the Dye Colors check box.***

Item	Description
❺	This ***Display Area*** graphically displays the opened data.
❻	 ***Fragment Data*** Displays the data that has been analyzed for the active sample. ***Fragment List*** Displays the fragment list from the analyzed data for the active sample. ***Analyzed Data*** Displays the analyzed data for the active sample. ***Current*** Displays the current data for the active sample. ***Voltage*** Displays voltage data for the active sample. ***Compare*** Used to visually compare the active fragment data set against up to seven additional fragment data sets.
❼	The ***Status Bar*** displays information concerning the current selection.

Menu Bar Options

The menu bar has the options shown in Figure 49. Tables 44 through 50 provide descriptions of the menu options.

Figure 49: Fragment Analysis Module, Menu Bar Options

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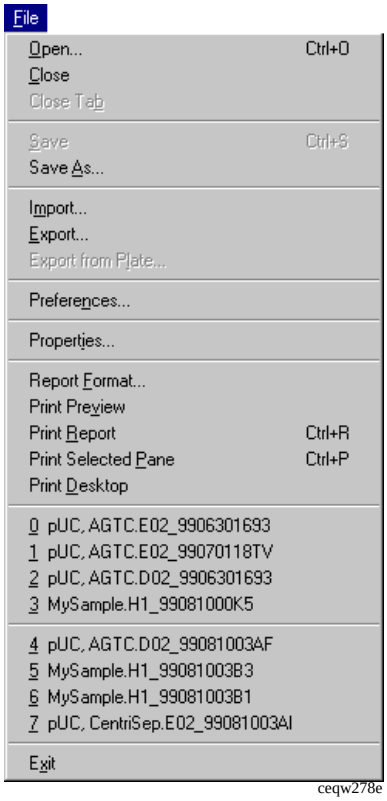
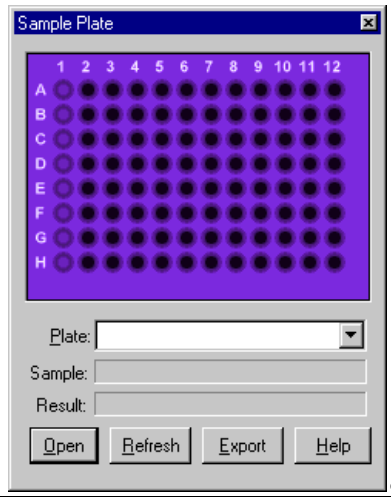
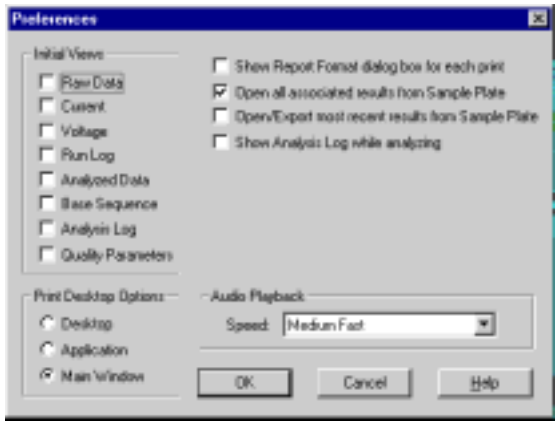


ceqw278e

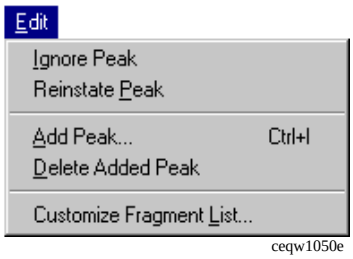
Table 44: Fragment Analysis Module, File Menu

Item	Description
Open	Used to open: Sample Data, Fragment Analysis Parameters, Optical Scan Data, Fragment Results, Standards, Locus Tags and Sample Plate Results.
Close	Closes the open sample.
Close Tab	Closes the active tab.
Save	Saves existing data.
Save As	Used to save data to a new name.
Import	Used to locate data to be imported. The Standard Chromatogram Format (*.scf) is the only available format for importing into the Fragment Analysis module.

Item	Description
Export	Used to export data in one of the following formats: • Standard Chromatogram Format Ver 3.00 (*.scf) • Standard Chromatogram Format Ver 2.10 (*.scf) • Tab Delimited ASCII Text (*.txt)
Export from Plate	Used to open or export samples from a specific sample plate. Multiple samples can be selected by using the **Shift** key. (The **Sample Plate** toolbar must be open to use this menu option.)  ceqw357e

Item	Description
Preferences	Used to: • view or change the panes displayed when the Fragment Analysis module is first opened. • show the **Report Format** dialog box when **Print Report** is selected from the **File** menu. • open all associated results that belong to a sample when a sample(s) is opened. • open/export the most recent results from a sample plate. • show the Analysis Log during analysis. • print the entire desktop. • print the application window, including all toolbars. • print only the displayed panes (**Main Window**).  ceqw1046e
Properties	Displays the current database, modification date as well as providing other information.
Report Format	Used to specify the format of the report that will be generated for the active sample.
Print Preview	Used to display a facsimile of a hardcopy printout of the selected sample plate.
Print Report	Used to print the report.
Print Selected Pane	Used to print the selected pane.
Print Desktop	Used to print the desktop, application or main window as defined in the **Preferences** dialog box.
Recent Samples	Lists the most recently opened samples. The first set of four is sample data and the second set of four is fragment results.
Exit	Closes the Fragment Analysis module.

Edit Menu

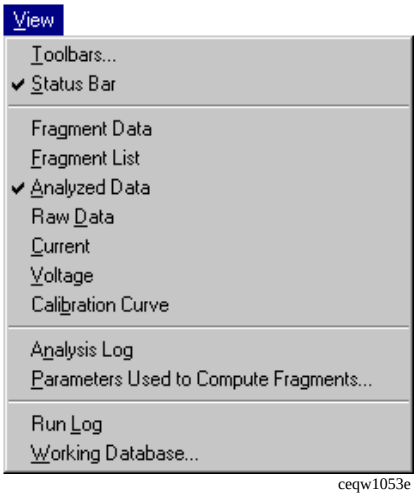


ceqw1050e

Table 45: Fragment Analysis Module, Edit Menu

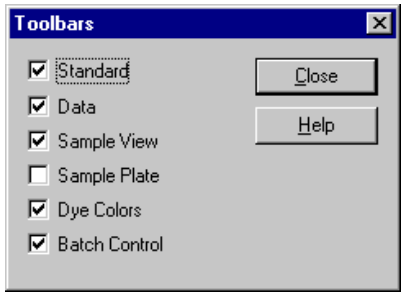
Item	Description
Ignore Peak	Used to ignore the selected fragment in the fragment list when the sample is reanalyzed. Fragment peaks that have been ignored are grayed-out in the fragment list. This option is only available in **Edit** mode and is used to ignore fragment peaks only.
Reinstate Peak	Used to reinstate a previously ignored fragment peak in the Fragment List.
Add Peak	Used to add a peak from the fragment data to the fragment list. If a trace is turned off, the peaks for the that trace are not eligible. An added peak will be graphically indicated by a plus sign (+) before the peak number in the fragment list. Any peak that lies between the upper and lower boundaries of the standard is eligible to be added.
Delete Added Peak	Used to delete a peak that has been added previously. Peaks generated by the size-calling algorithm cannot be deleted; they can only be ignored.
Customize Fragment List	Used to customize the display of information in the Fragment List.

View Menu



ceqw1053e

Table 46: Fragment Analysis Module, View Menu

Item	Description
Toolbars	Used to select which toolbars to display.  ceqw1054e
Status Bar	Toggles between displaying or not displaying the Status Bar.

Item	Description
Fragment Data	Displays Fragment Data. The Fragment Data is data that has been analyzed by the size-calling algorithm. Size labels will be shown for each valid peak.
Fragment List	Displays the Fragment List. The Fragment List contains information for each valid peak, such as size, height, area, locus and allele ID.
Analyzed Data	Displays Analyzed Data. Analyzed Data is pre-processed data from which peaks are detected and fragment data produced.
Raw Data	Displays Raw Data. Raw Data is data that has been collected directly from the CEQ instrument.
Current	Displays Current Data for the capillaries.
Voltage	Displays Voltage Data for the instrument.
Calibration Curve	Displays the **Calibration Curve** dialog box containing the calibration curve produced for that sample.
Dye Spectra	Displays the **Dye Spectra** dialog box containing the dye spectra values for the current analysis.
Analysis Log	Displays the Analysis Log for the analysis of the current sample.

Item	Description
Parameters Used to Compute Fragments	Used to: • View parameters used to perform the size-calling operation on the active sample. This dialog box is read-only. 
Run Log	Displays the messages received from the instrument during the sample run.
Working Database	Displays the name of the database in use.

Tools Menu

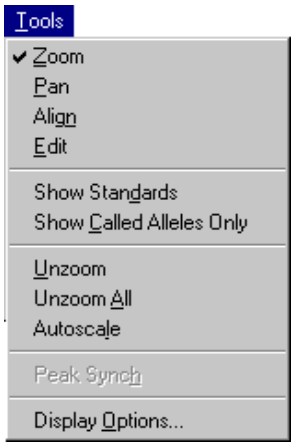


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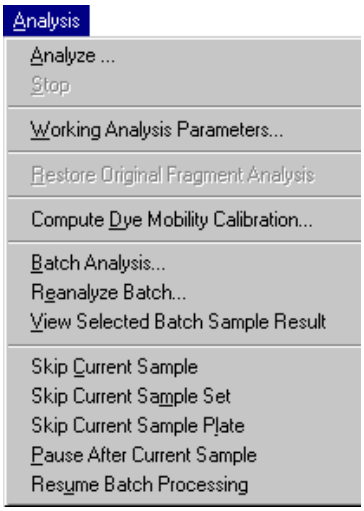
Table 47: Fragment Analysis Module, Tools Menu

Item	Description
Zoom	Used to magnify a specific area of data in the display.
Pan	Used to move raw, analyzed or fragment data in the X and Y direction.
Align	Used to visually align fragment sizes and peaks in the fragment data pane.
Edit	Used to ignore fragment peaks, reinstate ignored fragment peaks, add fragment peaks and delete added fragment peaks.
Show Standards	Used to view the sizes of standard traces in the fragment data pane and standard fragments in the fragment list.
Show Called Alleles Only	Used to display only the peaks that have been recognized as called alleles. Note ***If Show Standards is checked, the standard peaks will still appear in the Fragment List.***
Unzoom	Used to undo one zoom level.
Unzoom All	Used to undo all zoom levels.
Autoscale	Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this item to turn autoscaling on or off.
Peak Synch	With both the Fragment Data and Fragment List panes open, synchronizes the peak in the Fragment Data display with the highlighted fragment in the Fragment List.

Item	Description
Display Options	Invokes the **Display Options** dialog box used to modify any or all of the following parameters: • Title • X Axis Options • Y Axis Options • Dye Traces • Current Traces • Colors See immediate changes in the selected pane by clicking on **Apply**.  ceqw1094e Note ***Double-clicking the left mouse button in the selected pane, also brings up this dialog box.***

Analysis Menu

Table 48: Fragment Analysis Module, Analysis Menus



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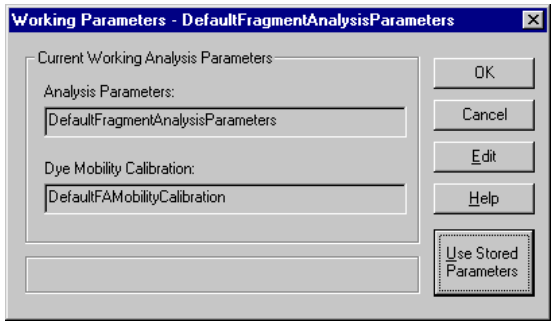
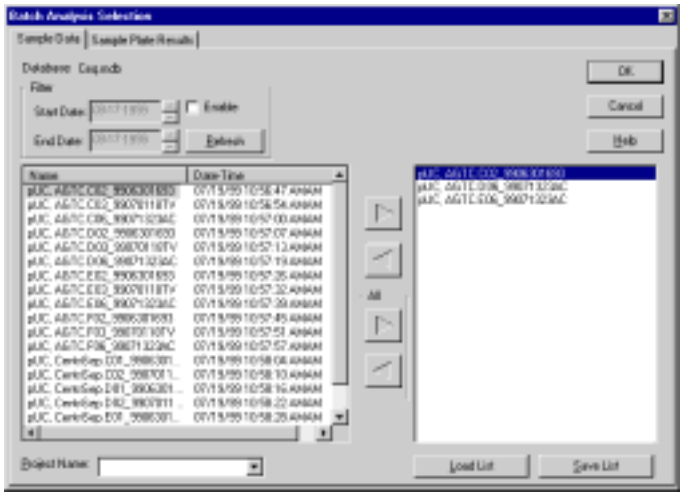
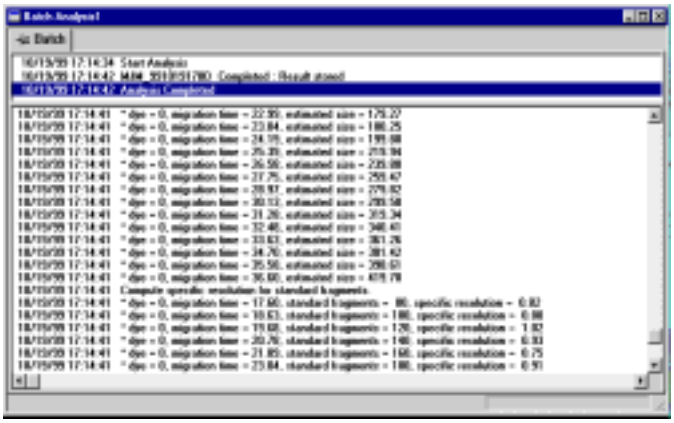
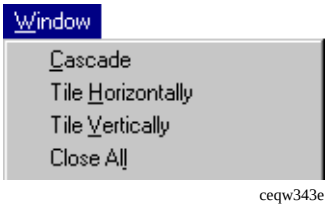
Item	Description
Analyze	Used to: • view the current working analysis parameters. • edit the working analysis parameters (maintained in memory for the duration of the application session). • select a previously saved parameter set to be used in the analysis. • begin the analysis when the **OK** button is selected.  ceqw1096e
Stop	Terminates the executing analysis.
Working Analysis Parameters	Used to view and/or change the analysis parameters to be used for subsequent analyses.
Restore Original Fragment Analysis	Used to return fragment data display and fragment list to its original state (losing all edits). If this option is grayed-out, it is not available.
Compute Dye Mobility Calibration	Used to create a new dye mobility calibration to be selected for use in subsequent analyses.

Item	Description
Batch Analysis	Used to analyze multiple Sample Data or Sample Plate Results sequentially. Invokes the following dialog box.  ceqw386e Once files have been transferred to the right-side list box and **OK** is selected, batch processing begins and a pane, similar to the one below, is displayed.  ceqw388e
Reanalyze Batch	After performing a batch analysis, use this option to perform the analysis again.
View Selected Batch Sample Result	After performing a batch analysis, use this item to view the results of a selected sample in the upper pane of the **Batch Analysis** window.

Item	Description
Skip Current Sample	While performing a batch analysis, use this item to pass over the *sample* currently being analyzed. (This item is not available if **Sample Plate Results** was selected from the **Batch Analysis Selection** dialog box.)
Skip Current Sample Set	While performing a batch analysis, use this item to pass over the *sample set* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
Skip Current Sample Plate	While performing a batch analysis, use this item to pass over the sample *plate* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
Pause After Current Sample	Used to momentarily stop a batch analysis after the currently running sample has been analyzed. Use **Resume Batch Processing** to continue the analysis.
Resume Batch Processing	Used to resume a batch analysis after it has been paused.

Window Menu

Table 49: Fragment Analysis Module, Window Menu



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Item	Description
Cascade	Cascades the open windows.
Tile Horizontally	Tiles the windows in a horizontal orientation.
Tile Vertically	Tiles the windows in a vertical orientation.
Close All	Closes all currently active windows.
Arrange Icons	Automatically arranges the icons.

Help Menu

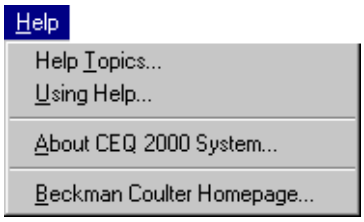

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Table 50: Fragment Analysis Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ 2000 System	Used to access software, instrument and system information.
Beckman Coulter Homepage	Used to access the Beckman Coulter Homepage on the Internet.

Toolbar Icons

There are six toolbars (Figure 50) used in the Fragment Analysis module: (1) Standard, (2) Data, (3) Sample View, (4) Dye Colors, (5) Batch Control, and (6) Sample Plate. Each icon of these toolbars corresponds to a common menu item. By default, all toolbars (except Sample Plate) are displayed when the Fragment Analysis module is opened. The tables below describe the functions of each of the toolbar icons.

Figure 50: Fragment Analysis Module, Toolbars

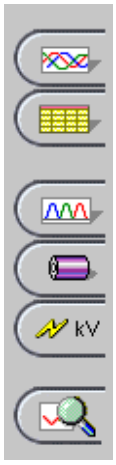
Standard Toolbar

(Table 51)

Data Toolbar

(Table 52)

Sample View Toolbar



(Table 53)

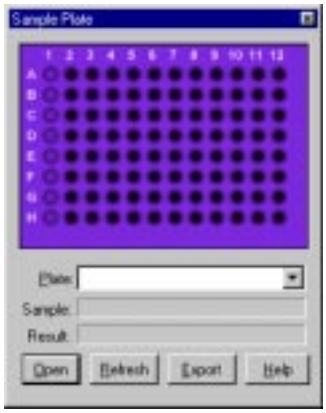
Dye Colors Toolbar

(Table 54)

Batch Control Toolbar

(Table 55)

Sample Plate Toolbar



(Figure 56)

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ceqw434e
ceqw1041e
ceqw1081e
ceqw1082e
ceqrf1015

Standard Toolbar

Figure 51: Fragment Analysis Module, Standard Toolbar

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Table 51: Fragment Analysis Module, Standard Toolbar

Icon	Description
	Open - Used to open: Sample Data, Fragment Analysis Parameters, Optical Scan Data, Fragment Results, Standards, Locus Tags and Sample Plate Results.
	Save - Saves existing data.
	Print - Used to print the report.
	Print Preview - Used to display a facsimile of a hardcopy printout of the selected sample plate.
	Print Desktop - Used to print the desktop, application or main window as defined in the **Preferences** dialog box.
	Working Analysis Parameters - Used to view and/or change the analysis parameters to be used for subsequent analyses.
	Analyze - Used to: • view the current working analysis parameters. • change the working analysis parameters (maintained in memory for the duration of the application session). • begin the analysis when the **OK** button is selected.
	Stop - Terminates the executing analysis.
	Restore Original Fragment Analysis - Used to return fragment data display and fragment list to its original state (losing all edits).
	Help Index - Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
	Context-Sensitive Help - Used to open the Help file related to a specific menu option.
	Ignore Peak - Used to ignore the selected fragment when the sample is analyzed. Fragment peaks that have been ignored are grayed-out in the fragment list and corresponding size labels in the fragment data are ignored.
	Reinstate Peak - Used to reinstate a previously selected ignored peak in the fragment list.

Icon	Description
	***Delete Added Peak*** - Used to delete a peak that has been added previously. Peaks generated by the size-calling algorithm cannot be deleted; they can only be ignored.
	***Add Peak*** - Used to add a peak from the fragment data to the fragment list. If a trace is turned off, the peaks for the that trace are not eligible. An added peak will be graphically indicated by a plus sign (+) before the peak number in the fragment list. Any peak that lies between the upper and lower boundaries of the standard is available to be added.
	***Customize Fragment List*** - Used to customize the fragment list. When the list is created following the analysis of a sample, the base attributes (Color, Std, Dye and Peak #) are always displayed. The Customize Fragment List option allows for the addition of other attributes assigned to the fragments in the list, depending on your needs.

Data Toolbar

Figure 52: Fragment Analysis Module, Data Toolbar

ceqw1082e

Table 52: Fragment Analysis Module, Data Toolbar

Icon	Description
	***Zoom Mode*** - Used to magnify a specific area of data in the display.
	***Pan Mode*** - Used to move fragment data in the X and Y direction.
	***Align Mode*** - Used to visually align fragment sizes and peaks in the fragment data pane.
	***Edit Mode*** - Used to ignore, reinstate, add or delete added fragment peaks. This option is used to edit fragment peaks and will not affect standard peaks upon reanalysis. Standard peaks are selected/deselected in the **Size Calibration** dialog box, accessed by selecting **Analysis** from the menu, then selecting **Analyze | Edit | Analysis Method | Define Model**.
	***Show Standards*** - Used to view the standard traces and standards in the fragment list. When deselected, fragments identified as members of the standard will be hidden from the fragment list and the size labels for the peaks hidden in the fragment data.
	***Show Called Alleles*** - When selected, only the peaks that have been recognized as called alleles will be displayed in the peak list from the unknowns peaks. The labels for fragment peaks that are not alleles will be removed in the fragment data.
	***Unzoom*** - Used to undo one zoom level.
	***Unzoom All*** - Used to undo all zoom levels.
	***Autoscale*** - Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this icon to turn autoscaling on or off.

Icon	Description
	Peak Synch - With both the Analyzed Data and Fragment List panes open, synchronizes the peak in the analyzed data display with the highlighted fragment in the Fragment List.
	Align - Used to align selected points of each of the displayed fragment data sets while in the **Compare** mode.
	Display Options -Invokes the Display Options dialog box.

Sample View Toolbar

Figure 53: Fragment Analysis Module, Sample View Toolbar

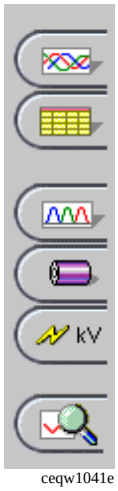

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Table 53: Fragment Analysis Module, Sample View Toolbar

Icon	Description
	Fragment Data - Displays the data that has been analyzed for the active sample. The Fragment Data is data that has been analyzed by the size-calling algorithm. Size labels will be shown for each valid peak.
	Fragment List - Displays the Fragment List from the analyzed data for the active sample. The Fragment List contains information for each valid peak, such as size, height, area, locus and allele.
	Analyzed Data - Displays the analyzed data for the active sample. Analyzed Data is pre-processed data from which peaks are detected and fragment data produced.

Icon	Description
	***Current*** - Displays the current data from the capillaries for the active sample.
	***Voltage*** - Displays the voltage data from the instrument for the active sample.
	***Compare*** - Used to visually compare the active fragment data set against up to seven additional fragment data sets.

Dye Colors Toolbar

Figure 54: Fragment Analysis Module, Dye Colors Toolbar

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Table 54: Fragment Analysis Module, Dye Colors Toolbar

Icon	Description
D1 (red)	The color red is the default color assigned to Dye #1 in the Fragment Data pane, and **D1** is the default name.
D2 (black)	The color black is the default color assigned to Dye #2 in the Fragment Data pane, and **D2** is the default name.
D3 (green)	The color green is the default color assigned to Dye #3 in the Fragment Data pane, and **D3** is the default name.
D4 (blue)	The color blue is the default color assigned to Dye #4 in the Fragment Data pane, and **D4** is the default name.

Batch Control Toolbar

Figure 55: Fragment Analysis Module, Batch Control Toolbar

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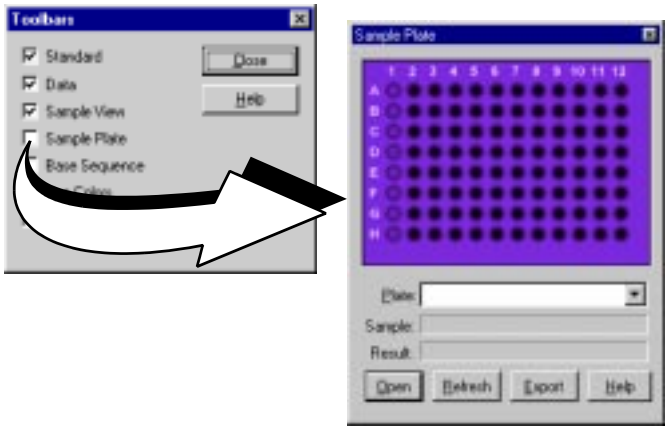
Table 55: Fragment Analysis Module, Batch Control Toolbar

Icon	Description
	***Batch Analysis*** - Used to analyze multiple Sample Data or Sample Plate Results sequentially.
	***Reanalyze Batch*** - After performing a batch analysis, use this option to perform the analysis again.
	***Stop Analysis*** - Terminates the executing analysis.
	***Pause*** - Used to momentarily stop a batch analysis after the currently running sample has been analyzed. Use **Resume Batch Processing** to continue the analysis.
	***Resume*** - Used to resume a batch analysis after it has been paused.
	***Skip Sample*** - While performing a batch analysis, use this item to pass over the *sample* currently being analyzed. (This item is not available if **Sample Plate Results** was selected from the **Batch Analysis Selection** dialog box.)
	***Skip Sample Set*** - While performing a batch analysis, use this item to pass over the *sample set* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
	***Skip Sample Plate*** - While performing a batch analysis, use this item to pass over the *sample plate* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
	***View Results*** - After performing a batch analysis, use this item to view the results of a selected sample in the upper pane of the **Batch Analysis** window.

Sample Plate Toolbar

Figure 56: Fragment Analysis Module, Sample Plate Toolbar



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Selected from the **View | Toolbars** menu option.

Used to specify samples to open or export. Select the desired plate from the drop-down menu. Valid samples appear as filled samples. Wells not specified as samples appear dark, or empty. Click on the samples to open or export.

To view the name of a sample and associated result, position the cursor over the sample. The sample and result names are displayed in the **Sample** and **Result** text boxes.

To Select contiguous samples, click on the first sample in the series, hold down the* Shift *key and then click on the last sample in the series. The selected samples are highlighted.

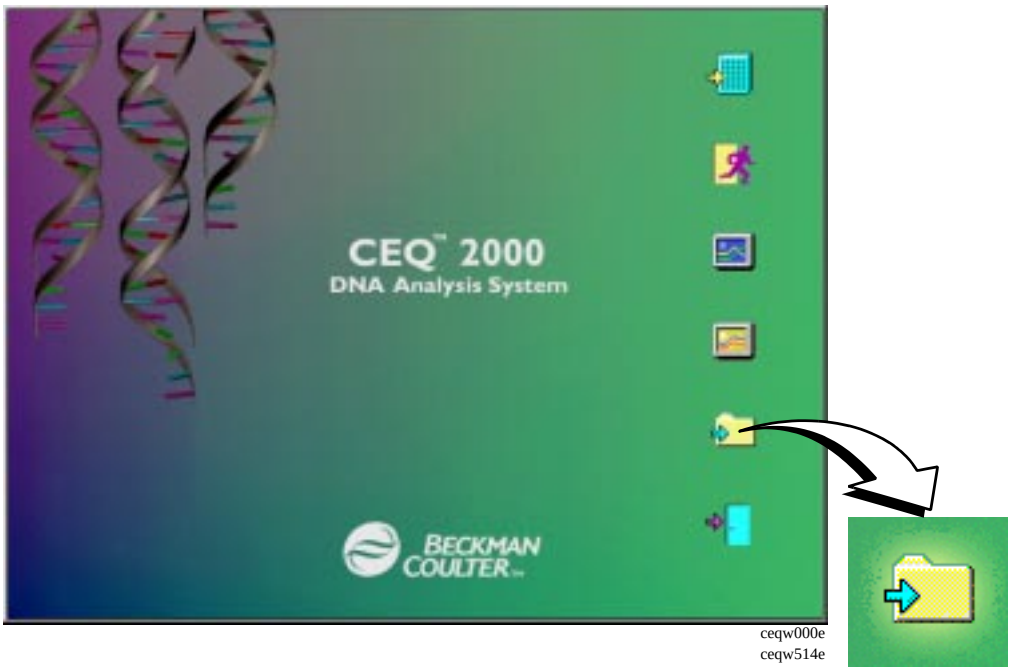
Data Manager Module

The Data Manager module is used to create, save, modify and print databases containing:

- Fragment Analysis Parameters
- Fragment Results
- Locus Tags
- Methods
- Optical Scan Data
- Sample Data
- Sample Plates
- Sample Plate Results
- Sequence Analysis Parameters
- Sequence Results
- Standards

Selecting the Data Manager *icon* from the Main Menu (Figure 57) executes this module.

Figure 57: Main Menu, Data Manager Icon



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Main Window

The main window of the Data Manager module is shown in Figure 58 and described in Table 56.

Figure 58: Data Manager Module, Main Window

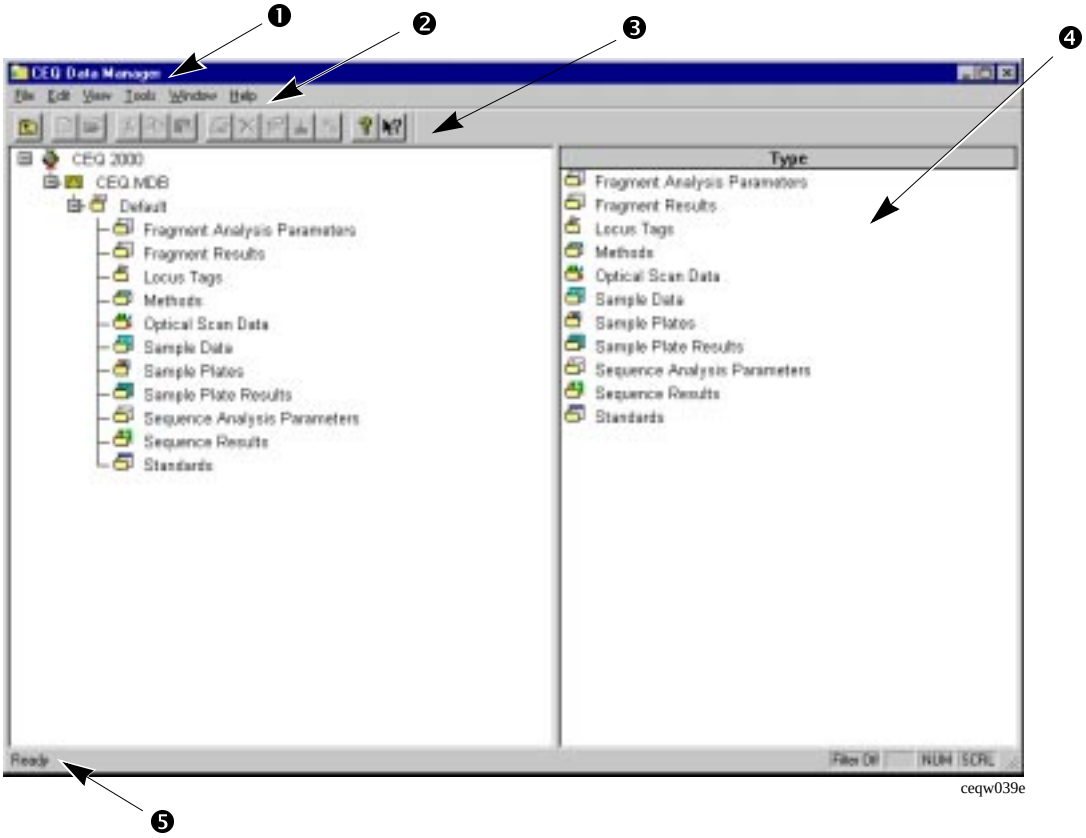

Table 56: Data Manager Module, Main Window Descriptions

Item	Description
❶	The ***Title Bar*** showing the module name (*CEQ Data Manager*).
❷	The ***Menu Bar*** is a list of the menu options (see ***"Menu Bar Options"*** on page 2-83).
❸	The ***Toolbar*** contains the icons that execute pre-defined functions (see ***"Toolbar Icons"*** on page 2-87).
❹	The ***Display Area*** lists the files contained in the selected node.
❺	The ***Status Bar*** displays information concerning the current selection.

Menu Bar Options

The menu bar has the options shown in Figure 59. Tables 57 through 62 provide descriptions of these menu options.

Figure 59: Data Manager Module, Menu Bar Items

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File Menu

Table 57: Data Manager Module, File Menu

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Item	Description
New	Used to create a new project.
New Database	Used to create a new database.
Open	Used to open and/or edit Fragment Analysis Parameters, Fragment Results, Locus Tags, Optical Scan Data, Sample Data, Sequence Results, Sample Plate Results, Methods, Standards, Sequence Analysis Parameters, or Sample Plates.
Print	Used to print a report of the selected item.
Print Setup	Used to define printer properties.
Delete	Used to delete items, projects and databases. Note ***The working database cannot be deleted!***
Rename	This item is used to rename items, projects and databases.
Properties	Displays the current database and modification date as well as providing other information concerning the selected item.
Import	Used to import a file in one of the following formats: • Standard Chromatogram Format v2.10 and v3.00 (*.scf) • CEQ (*.) • Tab Delimited ASCII Text (*.txt) A project must be selected to import items.

Item	Description
Export	Used to export a file in one of the following formats: • Standard Chromatogram Format v2.10 and v3.00 (*.scf) • CEQ (*.) • Tab Delimited ASCII Text (*.txt) • SEQ (*.seq) • FASTA (*.fasta) • PHRED (*.phd.1)
Set as Working Database	Defines the currently selected database as the default database.
Exit	Closes the Data Manager module.

Edit Menu

Table 58: Data Manager Module, Edit Menu

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Item	Description
Cut	Used to delete an item.
Copy	Used to duplicate an item, project or database.
Paste	Used to insert one or more copied or cut items, projects or databases.
Find	Used to locate a specific item, project or database.
Find Next	Searches for the next occurrence of the item or folder defined in the **Find** dialog box.
Select All	Selects all items under the highlighted node.

View Menu

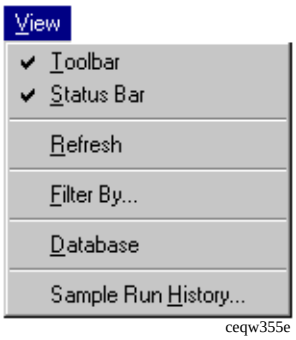

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Table 59: Data Manager Module, View Menu

Item	Description
Toolbar	Toggles between displaying or not displaying the toolbar.
Status Bar	Toggles between displaying or not displaying the Status Bar.
Refresh	Rebuilds the window display to reflect the most recent changes.
Filter By	Used to filter the list (in the right-hand side window) by the dates modified.
Database	Displays all items in the selected database.
Sample Run History	This dialog box is used to display a list of items that were run in a project during specified dates.

Tools Menu

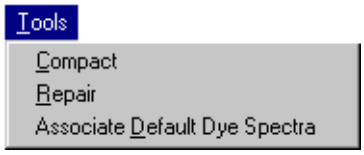

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Table 60: Data Manager Module, Tools Menu

Item	Description
Compact	Used to increase the storage efficiency of database data on the hard drive.
Repair	Performs functions similar to the MicroSoft “ScanDisk” and “Disk Defragmenter” System Tools on the selected database.
Associate Default Dye Spectra	Associates previously saved data with a new default dye spectra.

Window Menu

Table 61: Data Manager Module, Window Menu

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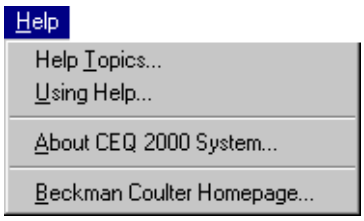
Item	Description
New Window	Opens a new window.
Cascade	Cascades the open windows.
Tile	Tiles the windows in a horizontal orientation.
Arrange	Automatically arranges the icons.
Close	Closes the currently active window.

Help Menu

Table 62: Data Manager Module, Help Menu



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Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ 2000 System	Used to access software, instrument and system information.
Beckman Coulter Homepage	Used to access the Beckman Coulter Homepage on the Internet.

Toolbar Icons

There is one toolbar (Figure 60) used in the Data Manager module. Each icon of this toolbar corresponds to a common menu item. Table 63 describes the function of each of the toolbar icons.

Figure 60: Data Manager Module, Toolbars

ceqw345e

Table 63: Data Manager Module, Toolbar

Icon	Description
	Up One Level **-** Moves the directory structure tree up one level.
	New **-** Used to create a new project or database.
	Open **-** Used to open and/or edit Fragment Analysis Parameters, Fragment Results, Locus Tags, Optical Scan Data, Sample Data, Sequence Results, Sample Plate Results, Methods, Standards, Sequence Analysis Parameters or Sample Plates.
	Cut **-** Used to delete items, projects and databases.
	Copy **-** Used to duplicate an item, project or database.
	Paste **-** Used to insert one or more copied or cut items, projects or databases.
	Print **-** Used to print a report of the selected item.
	Delete **-** Used to delete items, projects and databases.
	Properties **-** Displays the current database and modification date and other information concerning the selected data file.
	Compact Database **-** Used to increase the storage efficiency of database data on the hard drive.
	Repair **-** Performs functions similar to the MicroSoft “ScanDisk” and “Disk Defragmenter” System Tools on the selected database.
	Help Index - Used to select and print specific topics in the Help file and search for information by: topic, index entry and/or keyword.
	Context-Sensitive Help - Used to open the Help file related to a specific menu option.

Chapter Three 3

Operating the System

Chapter Overview

This chapter contains step-by-step procedures for all common tasks necessary to prepare and use the system on a daily basis. It also provides procedures to perform the tasks involved in analyzing/managing samples and sample data.

Set-Up & Quick-Start Procedures

Although the following procedures are independent for the set-up and quick-start of the system, perform the procedures below in the sequence provided.

Preparing Sample

1. Prepare sample for sequence or fragment analysis in accordance with the instructions contained in the chemistry kit.

Starting-Up the System

2. Set the PC power switch to the ON position and wait for Windows to start.
3. Set the CEQ instrument power switch to the ON position.
4. On the Desktop, select **Start | Programs | Beckman CEQ 2000 | Control Center**. The instrument will initialize and after several seconds the CEQ Main Menu will be displayed.

Creating a Database and Project Folder

Opening the Data Manager Module

5. From the CEQ Main Menu, click on the **Data Manager** icon and verify that the Data Manager window is displayed.

Creating a Database

6. In the Data Manager window, select **File | New Database**.
7. In the **New Database** dialog box, enter the name for this new database, select **Set as Working Database** (if desired) and select **OK**.

Creating and Naming a Project Folder

8. In the Data Manager window, highlight the desired database where the project will reside and select **File | New**.
9. Highlight the desired project and select **File | Rename**.
10. Enter a descriptive name for this project and press **Enter**.
11. Close the Data Manager module (**File | Exit**).

Setting up a Sample

Starting the Sample Setup Module

12. From the Shortcut Bar, click the **Sample Setup** icon and verify that the Sample Setup window is displayed.

Setting up the Sample Plate

13. Set up the sample plate by naming the desired cells.
14. Select a method from the drop-down menu at the bottom of the sample set.
15. Select **File | Save As**. Enter a name for the sample plate.
16. Select the project you just created from the **Project Name** drop-down menu and click **OK**.

Running a Sample

Starting the Run Module

17. From the Shortcut Bar, click on the **Run** icon and verify that the Run window is displayed.

Installing the Capillary Array

18. If necessary, perform the procedure "***Removing and Replacing the Capillary Array***" on page 3-63, and then return here.

Installing a Gel Cartridge

19. If necessary, perform the procedure "***Removing and Replacing a Gel Cartridge/Gel Pump Plug***" on page 3-70, and then return here.

Installing the Gel Waste Bottle

20. If necessary, perform the procedure "***Replacing the Gel Waste Bottle***" on page 4-5, and then return here.

Preparing Plates for a Run

21. Load samples into the sample plate.
22. Load buffer (250-300 µL) into the buffer plate.
23. Fill the wetting tray.

CAUTION **No more than one 96-well plate should be processed without replenishing the Wetting Tray.**

Periodically check the liquid level in the wetting tray. Liquid level should NEVER be allowed to rise into the eight cannula recesses of the wetting tray lid, nor drop below the fill level indicator line (9 mL minimum). The top surface of the wetting tray lid must remain clean and dry under any and all circumstances.

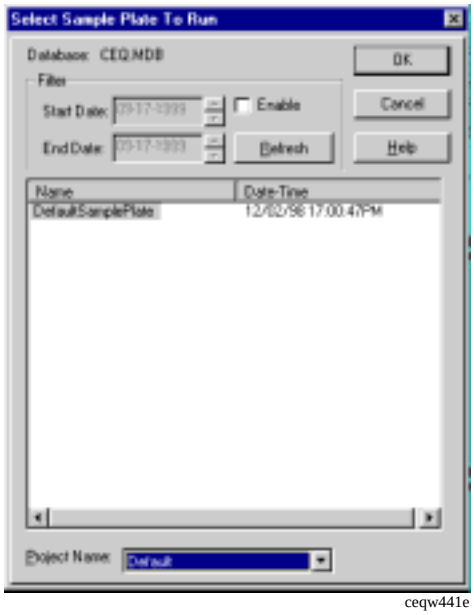
Loading the Wetting Tray, Sample Plate, and Buffer Plate

24. If necessary, perform the procedure "***Installing the Wetting Tray***" on page 3-56, and then return here.
25. If necessary, perform the procedure "***Loading the Sample Plate***" on page 3-58, and then return here.
26. If necessary, perform the procedure "***Loading the Buffer Plate and Evaporation Cover***" on page 3-59, and then return here.

Setting-Up and Starting the Run

27. Select **Run | Start Sample Plate** from the Run menu.
28. In the **Select Sample Plate To Run** dialog box (Figure 61), select the desired project and sample plate and click **OK**.

Figure 61: Select Sample Plate To Run Dialog



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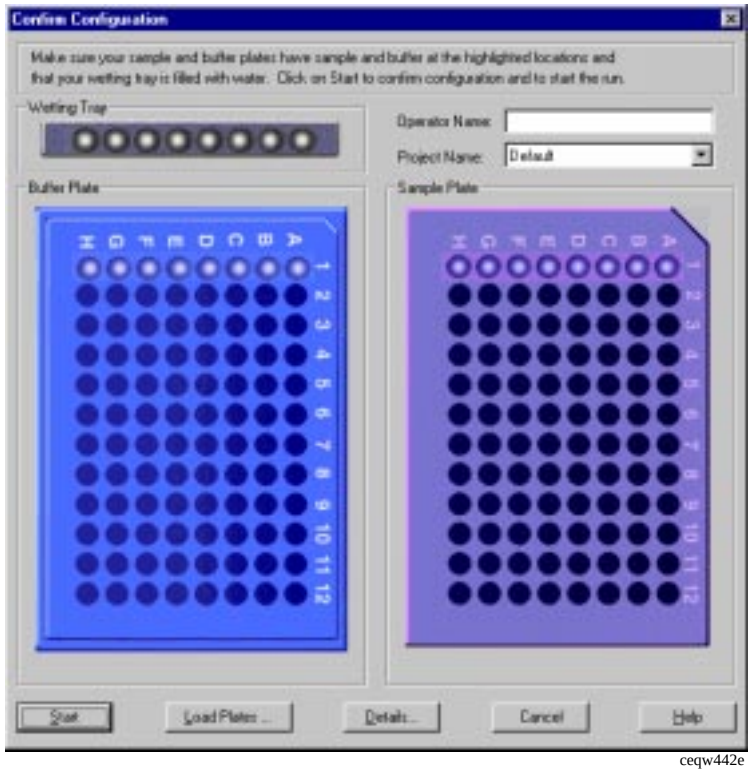
29. In the **Confirm Configuration** dialog box (Figure 62), verify the sample and buffer set locations and click **Start**.

Figure 62: Confirm Configuration Dialog



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Sample Setup Module Procedures

Opening the Sample Setup Module

From the Shortcut Bar, click on the **Sample Setup** icon and verify that the Sample Setup window is displayed.

Creating a New Sample Plate

To create a new sample plate, perform the following steps.

1. Select **File | New** from the menu.
2. Select (highlight) the cell or cells where the sample(s) will reside.
3. Enter the name of the sample(s) in the **Sample Name** text box and then press the **Enter** key.
   a. Assign a method to each sample set. To edit the method or create a new method, see "***Creating or Editing a Method***" on page 3-7, and then return here.
   b. To automatically analyze data after a sample set run, click on the **Analysis** tab and then on the **Automatic Analysis** check box in the **Analysis** window. The sample data will be analyzed with the parameter set selected from the drop-down menu. See ***"Assigning Parameter Sets"*** on page 3-8, and then return here.
   c. To automatically print a report after a sample set run, see "***Specifying Sample Plate Print Options***" on page 3-9, and then return here.
   d. To automatically export data after a sample set run, see "***Specifying Sample Plate Export Options***" on page 3-9, and then return here.
4. Select **File | Save As** from the menu.
5. In the **Save As** dialog box:
   a. S elect a **Project Name** from the drop-down menu**.**
   b. Enter a name for this plate in the name field.
   c. Select **OK** to save the plate.

Naming Samples

To name a single sample:

1. Select the cell where the sample resides in the plate.
2. Enter the name of the sample in the **Sample Name** text box and press the **Enter** key.

To name contiguous samples:

1. Select the cells where the samples reside in the plate by clicking and dragging the mouse cursor.
2. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.

To name all wells in the plate:

1. Click the **Select All**  icon.
2. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.

To name multiple samples that are not within the same sample set:

1. Select the first cell.
2. Hold down the Control key while selecting the additional samples.
3. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.

Assigning Methods

The method is the program of events the system uses to collect the data. The method controls the hardware, i.e. the temperatures, voltages and times, which work together to gather optimal data. The system comes with several methods that are optimized for the different software applications. The *Frag* methods are used to collect data for fragment analysis. The *LFR* methods are used to collect long fast read sequence data. The *DTCS* methods are used to collect standard sequencing data and the *Condition* method is used to condition a new capillary array in preparation for use.

To assign a method to one sample set:

At the bottom of the selected sample set, select a method from the drop-down menu.

To apply a method to multiple sample sets:

1. Highlight the desired sample sets.
2. Select **Edit | Auto-Fill Method Name** from the menu.
3. In the **Choose Method** dialog box, select a method from the drop-down menu.
4. In the **Auto Fill** area of the dialog box, select **Selected sample sets only** and then click **OK**.

To apply a method to all sample sets:

1. Select **Edit | Auto-Fill Method Name** from the menu.
2. In the **Choose Method** dialog box, select a method from the drop-down menu.
3. In the **Auto Fill** area of the dialog box, select **All sample sets** and then click **OK**.

Creating or Editing a Method

To create and/or edit a method, perform the following steps.

1. Select **Edit | Method** from the Sample Setup module menu.
2. Highlight the desired method in the **Choose Method to Edit** dialog box and click **OK**.
3. In the **Method - Capillary Temperature** dialog box:
   a. Enter a temperature between 35 and 65° C.
   b. Select the **Wait for Cap Temp** option (if desired).
   c. Click **OK** to exit the **Method** dialog box or continue with step 4 to make additional changes to the method.
4. Select **Denature** from the **Event** list and then:
   a. Enter a duration 0 and 180 seconds.
   b. Click **OK** to exit the **Method** dialog box or continue with step 5 to make additional changes to the method.
5. Select **Pause** from the **Event** list and then:
   a. Enter a time duration between 0 and 10 minutes.
   b. Select **OK**, and then **Save As**.
6. Select **Inject** from the **Event** list and then:
   a. Enter an injection voltage between 0.1 and 12.0 kV.
   b. Enter a time duration in seconds.
   c. Click **OK** to exit the **Method** dialog box or continue with step 6 to make additional changes to the method.
7. Select **Separate** from the **Event** list, and then:
   a. Enter the separation voltage between 0.1 and 12.0 kV.
   b. Enter a time duration in minutes.
   c. Click **OK** to exit the **Method** dialog box or continue with step 7 to make additional changes to the method.
8. In the **Save As** dialog box:
   a. Select a **Project Name** from the drop-down menu.
   b. Enter a name in the name field.
   c. Click **OK**.

Long Fast Read Procedures

To set up a sample plate for long fast read sequencing, perform the following steps.

1. Select the LFR-1 method for the sample sets that will perform long fast read sequencing. If you wish to edit the method or create a new one, it is recommended that you used a voltage between 4.0 and 5.8 kV and a temperature between 35 and 55°C.
2. If the longest fragments migrate past the detector prior to the end of data collection, the analysis may fail as the system attempts to analyze insignificant baseline data. If this is the case, try checking the **PCR Product** check box so the system will attempt to find the end of significant data. If the analysis still fails, enter an **Analysis Stop** time to force the analysis to only use data prior to the stop time. This will prevent the system from analyzing insignificant data.

Assigning Parameter Sets

Parameter sets define the conditions specific to an experiment used in data processing. *Sequence Analysis Parameter Sets* define the start and end times for the analysis of raw data, the threshold below which bases will be called as 'N's, and the information necessary to detect the start of data to be analyzed: the delay between the detected start of data and the start of data analysis, the signal to noise ratio, the minimum duration, and the threshold above which data will be considered peaks. *Fragment Analysis Parameter Sets* define the Locus Tags (DNA regions) of interest, the alleles found within those locus tags, and the standard to be run with the sample.

For sequence analysis, select a Sequence Analysis Parameter Set. For fragment analysis, select a Fragment Analysis Parameter Set. The selected parameter set can also be edited from this window.

To assign an analysis parameter set to one sample (well):

1. Highlight the desired sample.
2. Select the parameter set from the drop-down menu in the **Analysis** tab.

Do not mix sequencing and fragment analysis parameter sets.

To assign an analysis parameter set to one sample set:

1. Highlight the desired sample set.
2. Select the desired parameter set from the drop-down menu in the **Analysis** tab.

To apply an analysis parameter set to multiple sample sets:

1. Highlight the desired sample sets.
2. Select the desired parameter set from the drop-down menu in the **Analysis** tab.

Turning Cell Coordinates On or Off

To view or hide cell coordinates, select **View | Cell Coordinates** from the menu. (When checked, coordinates are displayed. When unchecked, coordinates are not displayed.)

Using Property Sets

1. To apply a property set to a sample(s), highlight the desired sample(s), click on the **Notes** tab and then on the **Edit Sample Properties**  button.
2. In the **Sample Property Sets** dialog box, highlight the desired property set (in the right-side list box) and then select **OK**.

Specifying Sample Plate Print Options

To define the print options and report format of the currently open sample plate, perform the following steps.

1. With a cell highlighted, select the **Analysis** tab at the bottom of the window.
2. Select the **Print Report** check box to print a report immediately after completion of the run.
3. Select the **Edit Print Format For Plate** button.
4. In the **Report Format** dialog box, verify or change the **Printer**, **Page Layout** and **Copies** options.
5. In the **Sample Elements** area of the **Report Format** dialog box, select each element to be printed.
6. If desired, select the **Options** button and verify or change the **Raw Data**, **Sequence Analysis Result Data**, **Fragment Analysis Result Data, Base Sequence Grouping** and **Current Trace Options** selections in the **Print Options** dialog box and then click **OK**. When printing Sequence Result Data, the Fragment Analysis Result Data option is not applicable. When printing Fragment Result Data, the Sequence Analysis Result Data and the Base Sequence Grouping options are not applicable.
7. If desired, select the **Colors** button and verify or change the trace colors of the **Raw Data** and/or **Analyzed Data** and then click **OK**.
8. Select **OK** from the **Report Format** dialog box to save the changes and close the dialog box.

Specifying Sample Plate Export Options

To specify the type of file and the included elements for export, perform the following steps.

1. With a cell highlighted, select the **Analysis** tab at the bottom of the window.
2. Select the **Export Data** check box.

3. Select the **Edit Export Options For Plate** button.
4. Select the file type. Some sample elements are grayed-out, or automatically checked when the different file types are selected. If the element is grayed-out and unchecked, the option is not available for that file type. If the element is grayed-out and checked, that item is automatically exported. These options differ for sequence data and fragment data.
5. In the **Sample Elements** area of the **Export** dialog box, select the type(s) of data to be exported (**Header, Raw Data**, **Result Data, Result Output,** and/or **Quality Parameters**).
6. In the **Options** area of the **Export** dialog box, select the desired file naming options.
7. Locate the target folder and then click **OK**.

Run Module Procedures

Defining System Preferences

To define the system preferences, perform the following steps.

1. Select **File | System Preferences** from the menu.
2. In the **System Preferences** dialog box:
   a. Enter the **System Name** and **Operator Name**.
   b. Select the **Project Name**.
   c. Define **Dye Names** for the Fragment Analysis module, if desired.
   d. Enable or disable the alarms and then select **OK**.

Running a Sample Plate

To run a sample plate that has been created and saved, perform the following steps.

1. Select **Run | Start Sample Plate** from the menu.
2. In the **Select Sample Plate To Run** dialog box, select a **Project Name** from the drop-down, highlight the desired sample plate and then select **OK**.
3. Load the wetting tray, sample plate and buffer plate if necessary.
4. In the **Confirm Configuration** dialog box, verify the sample position in the plate and then select **Start** to initiate the run.

Pausing a Sample Plate Run

To pause the currently executing sample plate, select **Run | Pause** from the menu.

Stopping a Sample Plate Run

To stop the currently executing sample plate, perform the following steps.

1. Select **Run | Stop System** from the menu.
2. In the **Stop System** dialog box:
   a. Select the desired **Stop Options** radio button.
   b. Select the **Save Collected Data** option (if desired).
   c. Select the **Perform Shutdown Method** (to purge the manifold and set capillary chamber temperature to 40°C), then select **OK**.

Setting or Changing Display Options

Setting or Changing Title Properties

To set or change the title of the Data Monitor display, perform the following steps.

1. Double-click the mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **Title** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing X Axis Options

To define the X Axis properties of the Data Monitor and Instrument Data displays, perform the following steps.

1. Double-click the mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **X Axis Options** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Y Axis Options

To define the Y Axis properties of the Data Monitor and Instrument Data displays, perform the following steps

1. Double-click the mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **Y Axis Options** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Dye Trace Displays

To set or change the dye trace properties, perform the following steps.

1. Double-click the mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **Dye Traces** tab.
3. Select the traces, to display or not display, under **Show Dye Traces**.
4. To change a color of any dye trace, click on the appropriate **Dye Colors** dye, select a color from the **Color** dialog box and then click **OK**.
5. When finished with Dye Traces, select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Current Trace Displays

To set or change the current trace options, perform the following steps.

1. Double-click the mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **Current Traces** tab.
3. Select the radio button for the current type to display under **Data**.
4. Select the capillaries, to display or not display, under **Show Current Traces** and then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Color Options

To define the color options of the window, the current traces, the voltage trace or the optical scan trace, perform the following steps.

1. Double-click the mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **Colors** tab.
3. Change any item as desired and then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Changing Raw Data Colors

To display the Raw Data Colors toolbar, select View | Toolbars from the menu. Select the desired toolbar options and click Close.

To change the colors of the raw data traces, perform the following steps.

1. On the **Raw Data Colors** toolbar, click on the trace color to change (**A**, **C**, **G** or **T** for Sequence Analysis and **D1**, **D2**, **D3**, and **D4** for Fragment Analysis).
2. In the **Color** palette dialog box, select the new color and then click **OK**.

Magnifying (Zooming) Data

To magnify data in the Data Monitor display, perform the following steps.

1. Click on the upper left-hand point of the area to be magnified, drag the mouse cursor down to the lower right-hand point of the area and then release the mouse button.
2. Repeat this procedure to increase magnification.

Viewing the Last Analysis Performed

To view the last analysis performed on the CEQ 2000 System, perform the following steps.

1. With a sample plate running (and with **Automatic Analysis** selected), select **Tools | View Last Analysis** from the menu.
2. In the **Sample Plate** dialog box, select a sample or multiple samples and then click **Open**. If the last analysis was a sequence analysis, the Sequence Analysis module will be launched and the sequence analyzed data displayed. If the last analysis was a fragment analysis, the Fragment Analysis module will be launched and the fragment data displayed.

Data Analysis Procedures

This section contains procedures related to both Sequence Analysis and Fragment Analysis. The ***Common Analysis Procedures*** section contains procedures that are shared by both modules. Any procedures that are specific to an analysis module are found in their respective sections, ***Sequence Analysis Procedures*** or ***Fragment Analysis Procedures.***

Common Analysis Procedures

Analyzing Raw Data

To analyze raw data in either the Sequence Analysis or Fragment Analysis modules, perform the following steps.

1. Select **File | Open** from the menu bar of the desired analysis module.
2. In the **Open** dialog box, select the **Sample Data** tab, highlight the desired sample name and then select **OK**. Make sure that you select data appropriate to the type of analysis you are performing.
3. Select **Analysis | Analyze** from the menu.
4. From the **Working Parameters** dialog box, make desired changes to the parameter set, then select **OK**.

Reanalyzing Data

To reanalyze data, perform the following steps.

1. With the desired sample data open, select **Analysis | Analyze** from the menu.
2. To select a new analysis parameter set:
   a. Select **Use Stored Parameters** from the **Working Parameters** dialog box.
   b. Choose the desired parameter set in the **Select Analysis Parameters** dialog box, then click **OK**.
3. To edit the currently selected parameter set:
   a. Select **Edit** from the **Working Parameters** dialog box.
   b. Make the desired changes to the parameter set in the **Analysis Parameters Editor** dialog box.
   c. Click **OK**, or click **Save As** to save the parameter set to a new name.
4. Click **OK** in the **Working Parameters** dialog box. The system will perform the reanalysis using the newly selected parameter set or the changes made to the previously selected set.

To keep the results from the previous analysis, "pin" the results tab by clicking on the pin icon . Clicking on the pin toggles between pinning and unpinning the results. Otherwise, the results will be overwritten by any subsequent reanalyses.

Viewing Parameters Used to Compute Sequence/Fragments

To view the parameters used to compute a sequence/fragment result, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sequence/Fragment Results** tab, highlight the desired result name and then select **OK.**
3. Select **View | Parameters Used to Compute Sequence/Fragments** from the menu. The **Analysis Parameters Editor** dialog box is displayed. The parameters used to process the open results are displayed in read-only fields.

Performing a Batch Analysis

To analyze more than one sample simultaneously, perform the following steps.

1. Select **Analysis | Batch Analysis** from the menu.
2. In the **Batch Analysis Selection** dialog box:
   a. Select a **Project** name from the drop-down menu at the bottom of the dialog box.

b. Select the appropriate tab: **Sample Data** or **Sample Plate Results**.
c. Select **Enable** and enter a filtering start and stop date, if desired.
d. Highlight a sample name and click the right arrow to move the sample name to the right-side listbox. Continue highlighting sample names and moving them to the right-side listbox until you have selected all the samples for analysis.
e. Select **OK**.

3. In the **Working Analysis Parameters** dialog box, edit the parameters, if necessary, then select **OK**.

Reanalyzing a Batch

To reanalyze a batch, perform the following steps.

1. After performing a batch analysis, select **Analysis | Reanalyze Batch** from the menu.
2. Select a new parameter set or edit the currently selected set from the **Working Analysis Parameters** dialog box to obtain different results.
3. Select **OK** to start the reanalysis.

To keep the results from the previous analysis, "pin" the results tab by clicking on the pin icon . Otherwise, the results will be overwritten by any subsequent reanalyses.

Viewing the Selected Batch Result

To view the selected batch result after performing a batch analysis, click the desired sample in the upper pane of the Batch Analysis window and then select **Analysis | View Selected Batch Sample Result** from the menu.

Skipping the Current Sample Analysis in a Batch

To skip the currently executing analysis of a *sample*, while a batch analysis is running, select **Analysis | Skip Current Sample** from the menu.

Skipping the Current Sample Set Analysis in a Batch

To skip the currently executing analysis of a *sample set*, while a batch analysis is running, select **Analysis | Skip Current Sample Set** from the menu.

Skipping the Current Sample Plate Analysis in a Batch

To skip the currently executing analysis of a *sample plate*, while a batch analysis is running, select **Analysis | Skip Current Sample Plate** from the menu.

Pinning Results

Several analyses (single or batch) may be necessary before a given result is satisfactory. The CEQ 2000 Software provides the ability to either overwrite a previous result, or produce additional result tabs.

1. To overwrite a previous result, click the pin in the left-hand corner of the result tab to "unpin" the result. A reanalysis of that sample will overwrite the existing result.
2. To produce a new results tab, click the pin to "pin" the result. Upon reanalysis, a new tab containing the reanalyzed results will be created.

Panning Data

To pan back and forth in the data pane, perform the following steps.

1. Select **Tools | Pan** from the menu.
2. Click anywhere in the Raw, Analyzed or Fragment Data pane and drag the mouse cursor left or right to move the display.
3. Deactivate the Pan Mode by selecting **Tools | Zoom** from the menu or by clicking on the **Autoscale** button.

Magnifying (Zooming) Data

To magnify data, perform the following steps.

1. Click on the upper left-hand point of the area to be magnified, drag the mouse cursor down to the lower right-hand point of the area and then release the mouse button.
2. Repeat this procedure to increase magnification.

Aligning Bases/Fragment Sizes and Peaks

To align the bases in the Base Sequence pane with the peaks in the Analyzed Data pane (Sequence Analysis), or to align the fragment sizes in the Fragment List with peaks in the Fragment Data pane (Fragment Analysis), perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Results** tab, highlight the desired sequence or fragment results and then select **OK**.
3. To visually align the peak with the letter or fragment size, click the **Align Mode** icon, then click down on the apex of the peak for which you wish to view the alignment. (A hairline cursor is displayed through the apex of the peak to its corresponding base or fragment size designation.)
4. Click the **Zoom, Align** or **Edit** button to get out of the Align mode.

Synchronizing Result Data with Result Output

To synchronize the Analyzed Data or Fragment Data with the Base Sequence or the Fragment List, perform the following steps.

1. Access the appropriate Analysis module.
2. Open the desired analyzed data.
3. Select **Tools | Base Synch (Peak Synch)** from the menu, or click the **Synch**  icon.
4. Select a base or range of bases in the base sequence pane or fragment or range of fragments in the fragment list. The corresponding peak(s) will appear between two hairline markers in sequence analysis. In fragment analysis, the selected peak(s) is shaded.

Using the Compare Function

To use the compare function, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sequence Results** or **Fragment Results** tab, highlight the desired results name and then select **OK**.
3. Select **Tools | Compare** from the menu.
4. In the **Compare** dialog box, select the desired sample name(s) for comparison and then select **OK**. In the Sequence Analysis module, two data sets can be compared, while the Fragment Analysis module allows up to eight data sets to be compared at one time. The selected data will appear, one on top of the other, on a new **Compare** tab.
5. Click the **Compare or Fragment Synch** icon to synchronize the sets of data. All panes will be zoomed and panned simultaneously when the active pane is being operated on.
6. Click the **Align Mode** icon to align the data.
   a. Place the hairline in the desired pane and click on the desired alignment point. This pane will act as the control and will be identified by a cyan blue outline. All other pane(s) will align with the alignment point selected in this pane.
   b. Place the hairline in the other pane(s) and click on the alignment points in the other data set(s).
   - Click the **Align** icon. The data will be aligned.
7. To create a second **Compare** tab, click the  to pin the current pane, then click **Compare** again to select data for the next comparison.

Setting or Changing Display Options

Setting or Changing Title Properties

To set or change the title of a pane, perform the following steps.

1. Select **Tools | Display Options** from the menu.
2. In the **Display Options** dialog box, select the **Title** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing X Axis Options

To set or change the X axis properties of a pane, perform the following steps.

1. Select **Tools | Display Options** from the menu.
2. In the **Display Options** dialog box, select the **X Axis Options** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Y Axis Options

To set or change the Y axis properties of a pane, perform the following steps.

1. Select **Tools | Display Options** from the menu.
2. In the **Display Options** dialog box, select the **Y Axis Options** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Dye Trace Displays

To set or change the dye trace displays of a pane, perform the following steps.

1. Select **Tools | Display Options** from the menu.
2. In the **Display Options** dialog box, select the **Dye Traces** tab.
3. Select the traces, to display or not to display, under **Show Dye Traces**.
4. To change a color of any dye trace, click on the appropriate **Dye Colors** dye, select a color from the **Color** dialog box and then click **OK**.
5. When finished with Dye Traces, select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Current Trace Displays

To set or change the current trace displays, perform the following steps.

1. Select **Tools | Display Options** from the menu.
2. In the **Display Options** dialog box, select the **Current Traces** tab.
3. Select the radio button for the current type to be displayed.
4. Select the capillaries, to display or not display, under **Show Current Traces**.
5. Select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Color Options

To set or change the color options in a pane, perform the following steps.

1. Select **Tools | Display Options** from the menu.
2. In the **Display Options** dialog box, select the **Colors** tab.
3. Change any item as desired and then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Specifying Dye Colors

To specify the dye colors for the data traces, perform the following steps.

1. Access either the Sequence or Fragment Analysis module.
2. On the **Dye Colors** toolbar, click on the well corresponding to the trace letter (for Sequence Analysis) or the dye (for Fragment Analysis).
3. In the **Color** palette dialog box, select the new color and then click **OK**.

Importing Data

To import data into either a database or an analysis module, perform the following steps.

1. When importing data into a database, first highlight the project in the database to be the destination for the imported items.
2. Select **File | Import** from the menu.
3. From the **Import** dialog box, locate the folder where the file resides, highlight the filename and then click **Open**.
4. When importing data into the Sequence or Fragment Analysis module, select the file type. The data will be displayed as a new pane.

If the data is not a file type that can be displayed in an Analysis module, the data will be unavailable for import.

Exporting Data

Sample Data or Result Data

To export sample data or result data from the Sequence or Fragment Analysis modules, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the appropriate tab (**Sample Data** or **Sequence/Fragment Results**), highlight the desired name and then select **OK**.
3. With the data displayed, select **File | Export** from the menu.
4. From the **Export** dialog box, locate the target folder and then click **Export**.

Sample Plate Results

To export sample plate results from the Sequence or Fragment Analysis modules, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sample Plate Results** tab, highlight the desired name and then select **OK**.
3. Select **View | Toolbars** from the menu. Select the **Sample Plate** check box, then click **Close**.
4. From the **Sample Plate** dialog box, select the sample(s) to export and then click **Export**.
5. From the **Export** dialog box, locate the target folder and then click **Export**.

Printing the Desktop

To print the desktop at any time, select **File | Print Desktop** from the menu then click **OK** in the **Print** dialog box. The desktop will be printed as defined in the **Preferences** dialog box.

Select File | Preferences to define Print Desktop Options.

Sequence Analysis Procedures

Viewing Sample Data

To view sample data, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the appropriate tab (**Sample Data**, **Sample Plate Results**, **Sequence Results**, **Sequence Analysis Parameters** or **Optical Scan Data**), highlight the desired item and then select **OK**.
3. Click on the **Sample View** toolbar icons to display the desired data.

Viewing and Editing Sequence Analysis Parameters

To view and/or edit the sequence analysis parameters, perform the following steps.

1. Select **File | Open** from the menu to select a stored parameter set.

To edit the working analysis parameters for the active sample set, select* Analysis | Working Analysis Parameters | Edit *and skip step 2.

2. In the **Open** dialog box, select the **Sequence Analysis Parameters** tab, highlight the desired filename and then select **OK**.
3. In the **Sequence Analysis Parameters Editor** dialog box:
   a. Enter a **Call Threshold** value above which peaks will be detected and their bases called. Peaks below this threshold will be deemed ambiguous and called as "N."
   b. Enter an **Analysis Start** time (the default of "0" will use the algorithm to find the start and stop times).
   c. Enter an **Analysis Stop** time.

d. Check **PCR Product** if the DNA in the sample was produced using PCR. This allows the CEQ algorithm to find the end of data specific to PCR generated fragments. (The algorithm will stop processing when it encounters the sudden drop of signal, usually seen with PCR generated fragments.)

An entered Analysis Start or Stop time will override any automatically determined start or stop time. In addition, if you enter a stop time that exceeds data collection time, analysis will terminate at the end of data collection.

4. If a value of "0" is entered for the **Analysis Start**, the information in the **Advanced** portion of the dialog box will be used by the base-calling algorithm to determine the start of data analysis.
   a. Enter the **% Threshold** necessary for data detection.
   b. Enter the **Delay** time between the start of data collection and the start of data analysis.
   c. Enter the **Signal to Noise** ratio for significant data.
   d. Enter the **Minimum Duration** the **% Threshold** (defined above) has to be exceeded without gaps greater than 21 seconds.
5. Select **OK** to save under the same name, or **Save As** to save as a new parameter set.

Pre-peak Reduction

Data collected from the CEQ 2000 DNA Analysis instrument may have small peaks ("pre-peaks") approximately one base prior to the peaks representing full-length DNA sequencing fragments. These pre-peaks appear in the same channel and have two confirmed sources:

- The presence of primers shorter than the full-length primer (N-1, N-2, etc. primers) are present during the sequencing reaction, and
- The presence of a run of a single base (e.g. polyA) in the template leads to a "copying" error.

To enable the system to attempt to identify and reduce the inclusion of pre-peaks in the analyzed data, check the **Pre-peak reduction** check box in the **Advanced** portion of the **Sequence Analysis Parameters Editor** dialog box.

If the primers used for the sequencing reaction are known to contain or suspected of containing N-1 primers as well as full-length primers, the Pre-peak reduction check box should be checked.

Long Fast Read Sequencing

Long fast read sequencing (LFR) allows the CEQ 2000 DNA Analysis System to sequence data up to approximately 700 bases in 100 minutes with 98% accuracy.

To use long fast read sequencing, perform the following steps.

Sample Setup Module

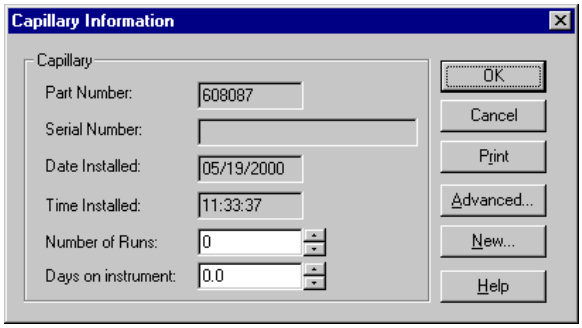
1. In the Sample Setup module, select the LFR-1 method for the sample sets that will perform long fast read sequencing. If you wish to edit the method or create a new one, it is recommended that you used a voltage between 4.0 and 5.8 kV and a temperature between 35 and 55°C.
2. If the longest fragments migrate past the detector prior to the end of data collection, the analysis may fail as the system attempts to analyze insignificant baseline data. If this is the case, try checking the **PCR Product** check box so the system will attempt to find the end of significant data. If the analysis still fails, enter an **Analysis Stop** time in the **Sequence Analysis Parameters** dialog box. This will force the analysis to only use data prior to the stop time and will prevent the system from analyzing insignificant data.

You can access this dialog box from the Sample Setup module by clicking Edit Parameter Set on the Analysis tab.

Run Module

3. Select **Replenish | Capillary Information** from the menu. Verify that the capillary array part number is 608087 (Figure 63). If this is not the information displayed, you must install the short capillary array and the Short Plenum Assembly, and update this information in the system.

Figure 63: Long Fast Read Capillary Information



Editing Bases

Inserting Bases in the Analyzed Data Pane

To insert bases in the Analyzed Data pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Click on an area *between* two bases.
3. From the **Insert Base** dialog box, select the desired base and then click **OK**. (The lower-case base is inserted in both the Analyzed Data and the Base Sequence pane.)

Inserting Bases in the Base Sequence Pane

To insert bases in the Base Sequence pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Place the mouse cursor *between* the two bases where you wish to insert the base, then type in the new base. (The lower-case base is inserted in both the Base Sequence and Analyzed Data pane.)

Changing Bases in the Analyzed Data Pane

To change bases in the Analyzed Data pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Click **on** a base in the analyzed data pane.
3. From the **Change Base** dialog box, select the desired base and then click **OK**. (The base is changed in both the Analyzed Data and the Base Sequence panes and will be lower-case to identify it as “edited.”

Changing Bases in the Base Sequence Pane

To change bases in the Base Sequence pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Highlight the base(s) to change in the base sequence text and then type in the new base(s). (The base[s] is changed in both the Base Sequence and Analyzed Data panes and will be identified as “edited” by being lower-case.)

The left and right arrow keys will move the cursor through the data in the Base Sequence pane. Bases can be highlighted by holding the* Shift *key down while using the left and right arrow keys to select the desired bases.

Deleting Bases in the Base Sequence Pane

To delete bases in the Base Sequence pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Highlight the base(s) in the base sequence text to delete and then press the **Delete** key (or use the Cut  icon). (The base[s] is deleted in both the Base Sequence and Analyzed Data panes.)

Specifying Base Grouping

To specify base grouping in the Base Sequence pane, perform the following steps.

1. Select **Tools | Base Spacing** from the menu.
2. From the pull-right, select the desired group spacing.

Restoring the Original Sequence Data

With a Results file open, select **Analysis | Restore Original Base Sequence** from the menu. This option will return the result data to its original state, losing all edits.

Using Quality Parameters

"Quality Parameters" are a measure of base call accuracy and can be used to determine which sections of a sequence are useful in subsequent sequence alignment, trimming purposes and assembly procedures. These values are also useful when examining a sequence either by eye or when using a sequence assembly program, such as *PHRAP,* or any primer design program.

In the CEQ 2000 Software, Quality Parameters include both Call Scores and Quality Values. Another value used to present information about analyzed DNA sequences is the Base Identity score.

The quality of called bases are assessed using two distinct calibrations: Call Scores and Quality Values.

Call Scores are one measure of the probability of correctness of a called base. They allow for more discriminating assessment of the bases called in the lower quality portion of the data (data with an error rate of *more* than one in one hundred). Call Scores aid in the evaluation of single-pass sequences.

Quality Values are another measure of the probability of correctness of a called base and are used specifically for sequences assembled in *PHRAP.* They allow for more discriminating assessment of the bases called in the higher quality portion of the data (data with an error rate of *less* than one in one hundred). Quality Values aid in the assembly of multiple sequences in *PHRAP* or any program that performs quality-weighted sequence alignment or primer design.

The Quality Parameters are assigned by a method that does not require knowledge of the true DNA sequence. These predicted probabilities of correctness of the called base, however, must correlate to observed error rates. The error rate is the actual number of errors in a section of DNA sequence divided by the number of bases in that section of DNA.

Both Call Scores and Quality Values may be viewed in a linear (0.00 to 1.00) or a logarithmic scale (0 to 100), as well as in line or bar graph format.

Errors in base-calling are often the result of misinterpretation of peaks in a region of the peak trace, but not in the peak itself. Examining the characteristics of the peaks in the vicinity of the erroneous peak reveals the indicators of error. The most effective parameters for detecting base-calling errors consider a window of the trace that includes several peaks flanking the one whose base-call is being assessed.

The CEQ 2000 Base Calling Software uses a combination of the following parameters to determine the Call Scores and Quality Values:

1. Peak spacing consistency - the ratio of the largest peak to peak spacing to the smallest peak to peak spacing in a given window of seven peaks.
2. Peak resolution - the number of bases between the current base and the nearest unresolved bases, multiplied by negative one (to force the parameter to have the right direction). This assesses variations in peak broadening and migration.
3. Uncalled peak to called peak ratio in a seven peak window - the ratio of the amplitude of the largest uncalled peak to the smallest called peak in a given window of seven peaks. This assesses the ability to call bases in that region of the trace.
4. Uncalled peak to called peak ratio in a three peak window - the ratio of the amplitude of the largest uncalled peak to the smallest called peak in a given window of three peaks.

“Call Scores” utilize parameters 1-3 as well as a ratio of base Call Scores for called events versus up to two neighboring uncalled events.

“Quality Values” utilize parameters 1 - 4 and allow error-prone base-calls to be identified with greater specificity.

Quality Parameters viewed in the logarithmic format have values ranging from 0 to 100. Use of a log-transformed error probability facilitates working with error rates in the range of most importance (very close to zero). The Quality Parameter, *q*, assigned to a base-call is defined by this equation:

$$q = -10 \times \log_{10}(p),$$

where *p* is the estimated error probability for that base-call. Thus, a base call with:

- A probability of error of 1/10 is assigned a value of 10.
- A probability of error of 1/100 is assigned a value of 20.
- A probability of error of 1/1000 is assigned a value of 30.
- A probability of error of 1/10,000 is assigned a value of 40.

High Quality Values correspond to high probabilities of correctness. Values above 20 indicate high quality bases and values above 30 indicate bases of very high quality.

Quality Parameters viewed in the linear format have values ranging from 0.00 to 1.00. Thus, a base-call with:

- A probability of error of 1/10 is assigned a value of 0.9.
- A probability of error of 1/100 is assigned a value of 0.99.
- A probability of error of 1/1000 is assigned a value of 1.00.

Either of the estimated probabilities of correctness can be viewed in the Analysis module when a Sequence Result is open. The next sections provide step-by-step instructions on the use of the CEQ 2000 Quality Parameters.

Viewing Quality Parameters

To display the Quality Parameters (Call Scores or Quality Values) in the Sequence Analysis window, perform the following steps.

1. Select **View | Quality Parameters**. The Quality Parameters plot will appear below the Analyzed Data pane.
2. Define the characteristics of the Quality Parameters plot using the procedure "***Setting or Changing Quality Parameter Display Options***"on page 28.

   Scrolling of the Quality Parameters plot is synchronized with the scrolling of the Analyzed Data.

To have the Call Scores plot always displayed when opening a sequence result, select Preferences in the File pull-down menu. Then enable Quality Parameters in Initial Views.

Viewing Quality Scores

To view the Quality Scores, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sequence Results** tab, highlight the desired result name and then select **OK**.
3. Select **View | Toolbars** from the Sequence Analysis menu.
4. In the **Toolbars** dialog box, select **Base Sequence**.
5. With the **Base Sequence** dialog box open, highlight the base(s) in the Base Sequence pane for which you wish to view the quality parameters. The quality parameters for the selected base(s) are displayed in the **Quality Scores** area on the right-side of the dialog box. The Call Score for a selected base is displayed in the **Call** text box. Below the Call Scores are the **Base Identity** scores. These scores, assigned to each trace by the base caller, indicate the likelihood that any base is A, C, G or T. For example, if a particular base has a higher score for A than C, G, or T, the likelihood is that the base is A as compared to the other three bases. The range for Base Identity scores is 0-254. Edited bases will have values of 255.

Setting or Changing Quality Parameter Display Options

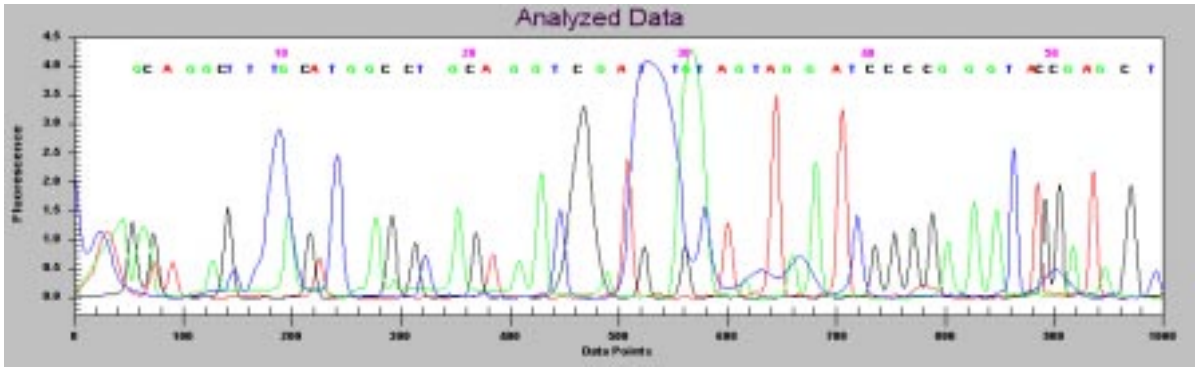
To set or change the quality parameter options in the pane, perform the following steps.

1. Select **Tools | Display Options** from the menu.
2. In the **Display Options** dialog box, select the **Quality Parameters** tab.
3. Click on the color well to change the color of the Quality Parameters plot.
4. Select either Call Score or Quality Values. The Call Score is a value from 0.0 to 1.0 that is a measure of the probability of correctness of the called base, with 1.0 being the highest probability of correctness. Quality Values are values that range from 1 to 100, and are another measure of probability of a called base. Quality Values are used specifically for sequences that will be exported into PHRAP for assembly.
5. Select either Linear or Log scale. A Linear scale is used to plot the values from 0.0 to 1.0 to reflect the values given by the CEQ 2000. The Log scale is used to plot the values from 0 to 100 to reflect the values used by PHRAP.
6. Select either Line or Bar graph.
7. Select **Apply** to continue or **OK** to close the **Display Options** dialog box.

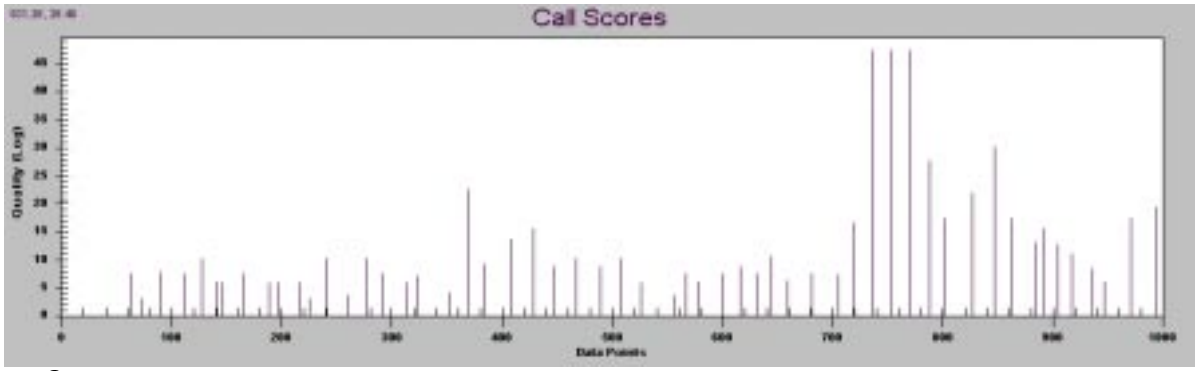
The characteristics of the Quality Parameters plot have been defined and the plot will appear below the Analyzed Data in the Analysis window. Scrolling of a Call Scores or Quality Values plot is synchronized with the scrolling of the Analyzed Data and both can be viewed simultaneously.

Figure 64: Analyzed Data (A) with Corresponding Linear (B) and Logarithmic (C) Call Scores Plots

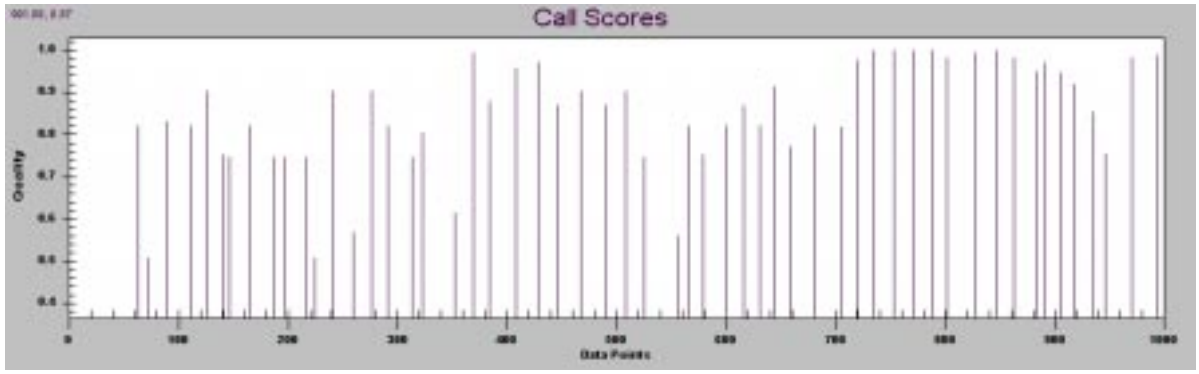
A:



B:



C:



Displaying Quality Parameters in a Report

1. To display the Quality Parameters in a report, go to the **File** menu and select **Report Format**.
2. In the **Sample Elements** section enable **Quality Parameters** then click **OK**.

Figure 65: Report Format Dialog Box

3. To view the Quality Parameters of an analyzed data set prior to printing, go to the **File** menu and select **Print Preview**. To exit the Print Preview option, click **Close**.
4. To print the Quality Parameters for that data set, go to the **File** menu and select **Print Report**. The Quality Values will be printed as numerical values and will appear as in the report below.

Figure 66: Quality Parameters Report

Project : Default
Sample : spa11.C3_04132276
Result : spa11.C3_9901201498

System : CEQ 2000 DNA Analysis

Operator : Manual analysis
Instrument : Unit11

Quality Parameters: spa11.C3_9901201498

Number	Base	A	C	G	T	CallScore	QualValue	Migration	DataPoint	Insert	Edit
1	G	0	0	243	0	0.82	9	37.09	63	No	No
2	C	0	157	0	0	0.51	9	37.15	72	No	No
3	A	253	1	0	0	0.83	7	37.34	90	No	No
4	G	0	0	247	0	0.82	9	37.33	111	No	No
5	G	0	0	246	1	0.90	10	37.41	127	No	No
6	C	0	236	0	0	0.75	6	37.49	141	No	No
7	T	0	0	4	212	0.74	5	37.81	146	No	No
8	T	0	0	20	234	0.82	9	37.90	165	No	No
9	T	0	0	0	250	0.74	5	38.00	187	No	No
10	G	1	0	246	0	0.74	6	37.76	196	No	No
11	C	1	238	0	0	0.74	6	37.86	216	No	No
12	A	148	0	4	0	0.51	9	37.98	225	No	No
13	T	0	0	1	253	0.90	12	38.25	241	No	No
14	G	0	0	156	81	0.57	12	38.07	259	No	No
15	G	0	0	251	0	0.90	10	38.16	276	No	No
16	C	0	251	3	0	0.82	9	38.24	291	No	No
17	C	0	246	2	0	0.74	6	38.34	312	No	No
18	T	0	0	10	244	0.80	9	38.63	322	No	No
19	G	0	0	253	1	0.61	10	38.54	352	No	No
20	C	0	250	0	1	0.99	31	38.62	369	No	No
21	A	251	0	3	0	0.88	13	38.75	383	No	No
22	G	1	0	253	0	0.96	18	38.83	408	No	No
23	G	0	0	254	0	0.97	17	38.93	428	No	No
24	T	0	0	0	254	0.87	12	39.21	445	No	No
25	C	0	254	0	0	0.90	12	39.12	467	No	No

The categories on the Quality Parameters Report are described in the following table:

Figure 67: Quality Parameters Report Categories

Number	the number of the called base
Base	the single letter designation of the called base (A, C, G, T or other IUB code)
A	base identity score assigned by the base-caller, ranging from 0 - 255
C	base identity score assigned by the base-caller, ranging from 0 - 255
G	base identity score assigned by the base-caller, ranging from 0 - 255
T	base identity score assigned by the base-caller, ranging from 0 - 255
Call Score	the calculated probability of correctness indicated on a linear (0.00 - 1.00) scale
Quality Value	the probability of correctness presented on a logarithmic (1 - 100) scale
Migration	the actual migration time (in minutes) in the raw data corresponding to the called base(s)
Data Point	the actual number of the data points in the analyzed data corresponding to the called base(s)
Insert	indicates a base that has been manually added to the base sequence
Edit	indicates that the base call has been manually changed

Preventing Alignment Problems

To prevent alignment problems caused by occasional high quality bases appearing in the lower quality 3’ end of a called sequence, it is suggested that you trim the 3’ end of the sequence.

Bases at the 3’ end of the sequence should be trimmed when:

- the average Call Score/Quality Value in a window of 25 bases falls below 0.75 - 0.8 on a linear scale and 6 - 7 on a log scale, **and**
- there are at least 5 bases within that 25 base window whose Call Score/Quality Value are less than 0.75 on a linear scale and 6 on a log scale.

End Trimming Before Export

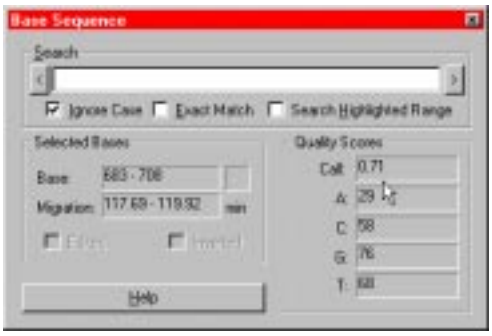
1. In the Sequence Analysis module, open the desired analyzed data set.
2. Enable the Base Sequence and the Quality Parameters by selecting those options in the **View** drop-down menu. The Quality Parameters plot and the Base Sequence dialog box will be displayed.
3. Access the **Quality Parameters** dialog box and set the plot characteristics to Call Score and Linear scale.

The end trimming information here is based on this format, but it can be viewed on a Quality Values, logarithmic scale plot as well. In that case, the threshold Quality Value for end trimming is 6.0.

4. Enable **Edit** mode by either clicking the right mouse button while the cursor is on the Base Sequence pane and selecting **Edit** from the pop-up menu that appears, or selecting **Edit** in the **Tools** drop-down menu.
5. In the Base Sequence pane, highlight a range of approximately 25 bases near the 3' end. Look at the average Call Score in the Base Sequence dialog box.

   If the average Call Score is below 0.75, the bases in that range need to be analyzed further to determine how many bases have Call Scores below 0.75.

Figure 68: Average Call Scores



6. View the Call Score data on a linear scale to determine if at least 5 bases in the 25 base window have a value of 0.75 or lower.

Double-click the right mouse button while the cursor is on the Call Scores plot. The **Display Options** dialog box will appear. Change the Y axis range maximum to 0.75.

The range maximum of the Y axis will now be 0.75, therefore, all peaks below this point (visible on the plot) will be below the threshold.

Figure 69: Analyzed Data with Corresponding Call Scores Plot and Base Sequence

7. On the Call Scores plot, count the peaks that fall below the threshold. Include only those that fall within the selected 25 base range, identified by the vertical pink markers in the Analyzed Data pane.

You can set the plot to display only the region between the pink markers by zooming in on that section. Place the cursor at the top of the Quality Parameter plot. Align the cursor with the left marker line then click and drag to the opposite corner of the desired section.

You can also check the Call Score of each base individually to determine how many within the 25 base range fall below 0.75. Highlight the first base in the desired sequence and use the arrow keys to move the cursor, or print a Quality Parameters Report.

8. If the selected data range meets both requirements for end trimming, highlight the bases from the beginning of the selected region to the end of the analyzed base sequence and press **Delete**.
9. If the selected data range does not meet both requirements, select another 25 base range closer to the 3' end and repeat steps five through eight.

End Trimming After Export

You can trim the ends of a called base sequence after export by exporting the analyzed data in either .FASTA, .PHRED or .txt format, then writing your own filtering program.

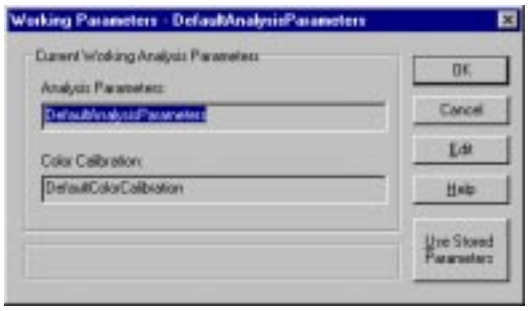
Setting the Call Threshold

In the CEQ 2000 Software, 'N's are assigned to regions of data where it is likely that an insertion or deletion exists, or where the identity of a called base(s) is ambiguous. The Call Threshold is used to specify the Call Score below which an 'N' will be assigned to the called base.

It is important to set the Call Threshold properly if you are exporting data to any program that can not use our Quality Values, such as BLAST or FASTA, or to most primer design programs.

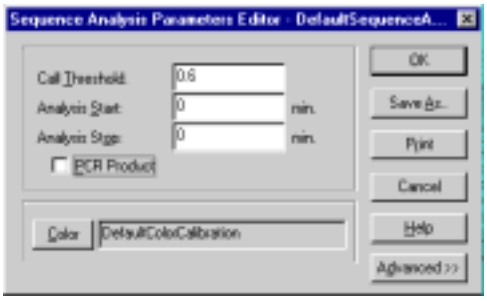
1. To define threshold parameters, select **Analysis | Working Analysis Parameters**.

Figure 70: Working Parameters Dialog Box



2. Click **Edit** in the **Working Parameters** dialog box.

Figure 71: Sequence Analysis Parameters Editor Dialog Box



3. To specify the threshold, enter the desired value, between 0.00 and 1.00, in the Call Threshold text box. A value of 0.99 will call all peaks as indeterminate (N's) except the high quality peaks. A value of 0.50 will call only the lowest quality peaks as 'N's. A value of 0.00 will not call any peaks as 'N's. The value of 0.60 is the recommended starting threshold. Subsequently, the threshold and/or the stringency of your alignment tool should be adjusted to suit your needs.

After setting the Call Threshold, the data must be reanalyzed.

If you had set a Call Threshold of 0.99, out of 100 bases called as 'N's, 99 of those would have been correct. The higher the Call Threshold, the higher the number of correct bases will be called as 'N's. Again, it is important to set the Call Threshold properly, as good quality data is lost when the Call Threshold is set too high.

4. Click **OK**. Any called bases or regions of data that have a Call Score below the specified threshold will be called as 'N's.

Viewing Color Calibrations

To view a color calibration for a sequence result, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sequence Results** tab, highlight the desired result name and then select **OK**.
3. Select **View | Parameters Used to Compute Sequence** from the menu.
4. In the **Parameters Used** dialog box, select **Color**.
   - To view the color calibration prior to the run, select the **Initial Values** radio button.
   - To view the color calibration after the run, select the **Final Values** radio button.

***This is a read-only dialog box. To make changes to the color calibrations, select* Analysis | Working Analysis Parameters | Edit | Color.**

5. To add a successful color calibration to the list of available color calibrations, click **Save As** in the **Color Calibration Editor** dialog box. Enter the desired name and click **OK**.

Computing a New Color Calibration

To compute a new color calibration, perform the following steps.

1. Select **Analysis | Working Analysis Parameters**.
2. In the **Working Parameters** dialog box, select the **Edit** button.
3. Click the **Color** button in the **Sequence Analysis Parameters Editor** dialog box.
4. Select the **Compute new color matrix** check box.
5. Select the **Use Final Values as Initial Values** check box to use the final values for the initial values for the computation (recommended if the peak resolution is good).

Viewing Data

To view data, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sequence Results** tab, highlight the desired results name and then select **OK**.
3. Select **View | Raw Data, Current**, **Voltage** or **Quality Parameters** (as desired) from the menu. By default, **Analyzed Data** and the **Base Sequence** panes are displayed.

Exporting Data to a Third Party Package

CAUTION **Verify that the third party analysis package will accept the data transferred, via a command-line, and that it is compatible with the file types provided by CEQ 2000 Software before attempting to perform this procedure.**

To export sequence data to a third party analysis package, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the appropriate tab, highlight the desired data and then select **OK**.
3. Select **Analysis | Third Party Analysis Setup** from the menu.
4. In the **Third Party Analysis Setup** dialog box:
   a. Specify the path and executable file of the third party analysis package.
   b. Select the appropriate **Export Data Format** radio button and then click **OK**.
5. Select **Analysis | Third Party Analysis** from the menu.
6. In the **Export** dialog box, enter the filename and then click **OK**. The data will be exported and the third party package launched with the exported data open.

Resolving File Name Conflicts

If the system encounters the same sample name as the destination, the name will normally be overwritten (this may happen if you choose to remove the plate position coordinates and the time/date stamp which ensures a unique name). If you check **Resolve Filename Conflicts**, in the **Export** dialog box, the system will increment the sample name(s) on export when it encounters a sample(s) with the same name(s). For example, if the system encounters the duplicate sample name of Fred on export, the system will rename the exported file to Fred_1.

You may automatically remove the plate position coordinates (*.A01, *.B01, etc.) and the time/date stamp from the sample name which is normally generated by the CEQ Sequencing software on export. To do so, check **Remove CEQ Tracking Suffix** in the **Export** dialog box.

Customizing Filename Extension

To supply your own custom filename extension (or none at all) to exported files, modify the CEQ.ini file as follows:

If there is no Export section in the CEQ.ini file, export any sample file and the Export section will be generated. Then modify as needed.

1. Find the section [EXPORT] and add/modify the appropriate entry.

   TXT_EXT for Text files, default is ".txt"

   SEQ_EXT for SEQ files, default is ".seq"

   FASTA_EXT for FASTA files, default is ".fasta"

   SCF_EXT for SCF files, default is ".scf"

   PHD_EXT for PHRED files, default is ".phd.1"

2. To have no file extension added, set the entry to nothing (make sure there are no spaces after the "=")

   SCF_EXT=

   Example Output FileName: SpaSample

3. To use a unique file extension, add or modify the entry with the desired file extension (do not place the leading dot ".", the system will add it).

   SCF_EXT=scf v3

   Output FileName: SpaSample.scf v3

4. To hide an entry and make the system use the default, either delete or place an ";" before it

   SCF_EXT=SCFV3

   Output FileName: SpaSample.scf

Specifying the Base Call Location

To specify the base call location, perform the following steps.

1. Open a Sequence Results file.
2. Select **Tools | Bases on Top** from the menu to toggle the bases from the top to the bottom of the Analyzed Data pane or vice versa.

Using Audio Enable

To enable the audio feature of the software, perform the following steps.

1. With a Sequence Results file open, select **Edit | Audio Enable** from the menu.
2. Enter text into the Base Sequence pane to hear the letter bases announced as they are typed.

To enter text in the Base Sequence pane,* Edit *mode must be enabled.

Using Audio Playback

To enable the audio playback feature of the software, perform the following steps.

1. With a Sequence Results file open, highlight a series of base sequence text.
2. Select **Edit | Audio Playback** to hear the announcement of the highlighted base sequence text.

To use this feature,* Edit *mode must be enabled.

Fragment Analysis Procedures

Viewing Sample Data

1. Select **File | Open** from the Fragment Analysis module menu bar.
2. In the **Open** dialog box, select the appropriate tab (**Sample Data**, **Sample Plate Results**, **Fragment Results**, **Fragment Analysis Parameters**, **Optical Scan Data**, **Standards** or **Locus Tags**), highlight the desired item and then select **OK**.
3. Click on the **Sample View** toolbar icons to display the desired data.

Viewing / Editing Fragment Analysis Parameters

The Fragment Analysis Parameter Set determines the analysis method for sizing peaks, the locus tags and the criteria used for identifying alleles.

1. Select **File | Open** from the menu in the Fragment Analysis module.

Fragment Analysis Pa rameter Sets can also be accessed and edited in the Sample Setup module.

2. In the **Open** dialog box, select the **Fragment Analysis Parameters** tab, highlight the desired name and then select **OK**.
3. The **Fragment Analysis Parameters Editor** is displayed. Use this dialog box to specify the parameters used in processing data and sizing fragments. The following sections, page 39 through page 45 describe these parameters in detail.

Using a Standard

A standard is a set of DNA fragments of known sizes that are run with a sample and are used to create the calibration curve. A size standard creates a list of fragments that should correspond to the standard run with the sample. When the standard is run, all the specified fragments should appear as peaks in the fragment data. The fragment sizes and constant velocity migration times of the associated peaks are used to calculate a calibration curve that relates size to migration times. This calibration curve is used to estimate sizes for the unknown fragments.

1. In the **Fragment Analysis Parameters Editor** dialog box, select the standard run with the sample from the **Standard** drop-down list.

Creating a New Standard

2. Select **File | Open** from the menu.
3. In the **Open** dialog box, select the **Standards** tab.
4. Highlight the desired standard and click **OK**.

The standard must be a system standard provided by Beckman Coulter.

5. In the **Size Standard** dialog box:
   a. Select the desired dye for the standard trace.
   b. Change the size for any fragment in the standard by double-clicking in the cell and entering the desired size between 0 and 30,000.
   c. Add a new fragment by clicking on the **New**  button and entering the desired size.
   d. Delete a fragment by selecting the desired fragment and clicking on the **Delete** button.
   e. Click **Save As** to save the parameters as a new Standard.

When a standard is changed and saved, the next time the new standard is opened, the **Size Standard** dialog box is read-only and that standard cannot be changed again. Additional changes are made from the system standards, and saved to another new name. Permanent changes cannot be made to the standards provided with the system.

Using the Standard Mobility Reference

The Standard Mobility Reference aids in determining the mobility of the peaks in the standard. The Standard Mobility Reference is a cubic polynomial fitted to constant velocity migration times as a function of fragment size. The use of a standard mobility reference minimizes the probability of detecting an inappropriate standard fragment.

The standard mobility reference is a calibration curve from an existing result with similar separation conditions. The curve must be a cubic polynomial fit, and the Y variable must be mobility for the Y axis in the curve fit.

1. In the **Fragment Analysis Parameters Editor** dialog box, select the desired standard mobility reference for the sample from the **Std Mobility Reference** drop-down list.
2. To save the parameters of the standard calibration curve as a Standard Mobility Reference to be used in subsequent analyses:
   a. Open the desired result.
   b. Select **View | Calibration Curve** from the menu. The **Calibration Curve** dialog box is displayed.
   c. Click the **Save As Standard Mobility Reference** button in the Calibration Curve dialog box.
   d. Enter the name of the standard mobility reference, the click **OK**.

   The new reference will be added to the Standard Mobility Reference drop-down list.

This standard mobility reference should be used for samples run under similar separation conditions and with the same standard.

Editing Locus Tags

A locus tag is used to query a locus of interest. The locus tag defines the fragment size range, the dyes to be used for the locus, the allele list for the locus and parameters that define valid alleles.

1. Select the Locus Tags to be used in the analysis by clicking on the locus tag names in the **Available** list, and using the arrow buttons to transfer them to the **Selected** list, and vice-versa.
2. To edit a locus tag, select the desired locus tag then click the **Edit Locus Tag** button.
3. Use the **Locus Tag Editor** dialog box to:

a. Enter the name of the locus tag using up to 128 characters (used in the fragment list).
b. Enter the GenBank Accession number using up to 128 characters (for documentation purposes only).
c. Select the minimum and maximum fragment size associated with the locus, between 1 and 30,000 nucleotides.
d. Enter the primer set name (for documentation purposes only).
e. Select the dye to be used for the primer set.
f. Specify whether the primer is labeled on the forward or reverse strands, or select **None** if you do not wish to document which strand is using the dye label (for documentation purposes only).
g. Enter the sequence for the primer using up to 128 characters (for documentation purposes only).
h. Click the **Allele List** button, if you wish to automatically identify alleles. The Allele List dialog box is displayed. Make the desired changes and select **OK** to return to the **Locus Tag Editor** dialog box.
i. Click **OK** to return to the **Fragment Analysis Parameters Editor** dialog box, or on **Save As** to save the changes to a new locus tag name.

4. Click on the **Analysis Method** tab to specify the following parameters for analysis:
   a. Enter the minimum peak height using the **Minimum Peak Height** spin control.
   b. Select the **Identify Allele** check box to have the system identify alleles as specified in the allele lists of the selected locus tags.
   c. Click **Define Model** to access the **Size Calibration** dialog box. Select the peaks from the specified standard, and select the model and the Y variable for the calibration curve to be used for the analysis.
   d. Click **OK** to return to the Analysis Method tab.
5. Click **Advanced** to display and edit the following advanced parameters:
   a. Select the desired Dye Mobility Calibration from the **Dye Mobility Calibration** drop-down list. If NoCorrection is selected, the system will not make any compensation for dye migration in the analysis of the fragments.
   b. Click the **Edit** button next to the selection to view and/or edit the Dye Mobility Calibration. The **Dye Mobility Calibration** dialog box is displayed.

WARNING **Do not change the values in the Dye Mobility Calibration dialog box unless instructed to do so by Beckman Coulter service personnel.**

   c. Specify the minimum rate of the signal increase on the leading edge of peaks in order for peaks to be detected by entering the desired value using the **Slope Threshold** spin control.
6. Click **OK** to enter any changes.
7. Click **Save As** to save the parameter set to a new name.

8. Click the **Print** button to print the contents of the analysis parameter set.
9. Click **Cancel** to exit the dialog box without entering any changes.

Setting the Slope Threshold

The slope of a peak is used to define the lowest peak size detected by the system. The slope is directly proportional to the size of the peak and can be used to detect peaks down to a certain size, while eliminating smaller peaks that are not of interest. The slope threshold is set relative to the baseline noise, as the baseline noise will vary for each channel and dye. Although only one slope threshold value is defined, the system will make adjustments for each dye, depending on the noise detected for that channel and dye.

The default value should be appropriate for most samples, however, if the results are not desirable, slight adjustments should be made.

To adjust the slope threshold to suit your data, perform the following steps, depending on the desired results.

1. If the peaks in a particular sample are small and not detected by the system during the previous analysis, return to the **Fragment Analysis Parameters Editor** dialog box to make these adjustments:
    a. Reduce the slope threshold by half of the currently entered value.
    b. Click **OK** to return to the Working Parameters dialog box.
    c. Click **OK** again to reanalyze the data.
    d. Assess the results to determine to effect of the modified slope threshold. Repeat steps a through d until the desired peaks are detected and analyzed by the system.
2. If the peaks in a particular sample are larger and the system has detected peaks you are not interested in, return to the **Fragment Analysis Parameters Editor** dialog box to make adjustments.
    a. Increase the slope threshold by half of the currently entered value.
    b. Click **OK** to return to the Working Parameters dialog box.
    c. Click **OK** again to reanalyze the data.
    d. Assess the results to determine to effect of the modified slope threshold. Repeat steps a through d until the desired peaks are detected and analyzed by the system.

Creating an Allele List

The **Allele List** dialog box is used to define the parameters of the allele, and to generate lists of alleles for the selected locus tag. The name of the associated locus tag is displayed at the top of the dialog box.

1. Click **Allele List** in the Locus Tag Editor dialog box to access the **Allele List** dialog box.
2. Select the desired **Repeat Unit Length**, from 1 to 255 nucleotides, using the spin control.
3. Enter the **Repeat Unit Sequence**, 1 to 255 nucleotides, using the spin control (optional).
4. Specify the criteria for identifying alleles by clicking on the **Allele ID Criteria** button. Make the desired selections, then click **OK** to return to the Allele List dialog box.
5. Select the number of repeats in the shortest allele, from 1 to 1000 nucleotides, using the spin control.
6. Specify the **Shortest Allele Size** using the spin control. The value entered here must be within the range set for the **Minimum** and **Maximum Fragment Size Range** in the **Locus Tag Editor** dialog box. If the value falls outside this range, a warning dialog box will appear.
7. Select the **Numeric** radio button to list the alleles numerically or the **Alphabetic** radio button to list them alphabetically.
8. Click the **Generate List** button to generate a new list or overwrite an existing list. The list is displayed in the **Allele List** box. The allele list displays the true size of the allele, the apparent allele size (corrected for dye migration), the number of repeats in the allele and an allele identifier used in the fragment list.

Editing the Allele List

Information in an **Allele List** can be entered or changed in the following ways:

1. Existing allele list information can be changed by double-clicking on the desired cell and entering new information.
2. Click the **New**  icon to add a new allele. An empty row is added to the allele list box. Enter the desired information.
3. Highlight the desired cell and click the **Delete**  icon to delete an allele.

Specifying Allele ID Criteria

From the **Fragment Analysis Parameters Editor** dialog box, select **Edit Locus Tag | Allele List | Allele ID Criteria**. The **Allele ID Criteria** dialog box is used to specify the criteria for identifying alleles.

1. Specify the size tolerance for calling alleles by entering the desired value, in nucleotides, using the **Allele Confidence Interval** spin control. The system will call the allele if it is within the bounds of the value entered.
2. Check the **Detect +/- A** and the **Use +A peak to call Allele** check boxes to identify the true allele as -A for the fragment list (the +A peak will be identified as the allele). Check the **Use +A peak to call Allele** check box, but not the **Detect +/- A** check box to identify the true alleles as +A for the fragment list (the -A peaks will not be identified).
3. Check **Search for stutter** to specify to the system to search for stutter.
4. Enter the number of repeats from 1 to 100 to specify the window around each allele in which stutter will be detected.
5. Check **Detect stutter shorter than allele** to detect stutter prior to alleles.
6. Check **Detect stutter longer than allele** to detect stutter after alleles.
7. Specify the percentage relative to the true allele below which detected peaks are considered stutters using the **Maximum stutter peak height** spin control.
8. Check **Detect spurious peaks** if you wish to remove spurious peaks which are below the **Maximum height for spurious peaks** threshold. Enter the desired spurious peak height threshold, from 1 to 100%, relative to the reasonable allele peak height as calculated by the system. Peaks above this threshold will be labeled as unknown alleles.
9. Click **OK** to enter any changes and close the dialog box. Click **Cancel** to exit the dialog box without entering any changes.

Defining the Size Calibration

From the **Fragment Analysis Parameters Editor** dialog box, select the **Analysis Method** tab, then **Define Model**. The **Size Calibration** dialog box is used to specify the peaks of the selected standard to be used in analysis, as well as the model and the Y variable parameters for the calibration curve.

To define the parameters of the size calibration and the resulting calibration curve, perform the following steps.

1. Select the peaks in the **Use** column that are to be used in determining the standard curve. Use the **Select All** button to select all peaks. Use the **Deselect All** button to deselect all peaks. Peaks that are deselected in this dialog box will be grayed-out in the fragment list and will have a “-” in the **Std** column.

2. Select the desired **Model** to be used to the analysis.

   The drop-down menu presents 5 choices: Linear, Quadratic, Cubic, Quartic and Local Southern. Each of the first four will construct a size calibration as a least squares polynomial fit using known fragment sizes and either average migration times or mobilities. The Local Southern method estimates the size of a sample fragment from its mobility and the mobilities of the four standard fragments closest in size.

3. If Linear, Quadratic, Cubic or Quartic was selected in step 2, the **Y variable** must also be defined. (If Local Southern is selected, mobility is automatically set as the Y variable.) Select either **Migration Time** or **Mobility** for the Y axis. The selection of Migration Time will use the average migration times and sizes of the standard fragments to construct a size calibration. The selection of Mobility will use fragment velocity in centimeters per second divided by separation potential in volts per centimeter with the sizes of the standard fragments to construct a size calibration.

4. Click **OK** to enter changes and return to the **Fragment Analysis Parameters Editor** dialog box. Click **Cancel** to exit the dialog box without entering changes.

Editing and Saving the Calibration Curve

To edit the calibration curve and/or save the calibration curve as a standard mobility reference, perform the following steps.

1. Select **View | Calibration Curve** from the menu. The **Calibration Curve** dialog box displays the calibration curve produced by the standard from the current analysis and is used to view or edit the calibration curve.

2. To edit the calibration curve:

   a. Click **View Model** in the Calibration Curve dialog box. The **Size Calibration** dialog box is displayed.
   b. Select/deselect standard peaks by checking/unchecking the check boxes in the list box.
   c. Click **OK** in the **Size Calibration** dialog box.
   d. Click **OK** in the **Calibration Curve** dialog box to recalculate the curve to include or exclude any removed or added standard peaks in the curve.

3. To select another model (the functional form of the curve) and the Y variable:

   a. Click the **View Model** button to display the **Size Calibration** dialog box.
   b. Select the model for the curve. If you select Local Southern, skip step c. The Local Southern automatically uses mobility as the Y variable in creating the calibration curve.
   c. Select the appropriate Y variable.
   d. Click **OK** to exit the **Size Calibration** dialog box.
   e. Click **OK** to recalculate the calibration curve and reanalyze the data using the new calibration curve.

If the active result is pinned  , a new result is displayed. If the active result is unpinned  , the new result will overwrite the previous result. To change the result from pinned to unpinned or vice-versa, click the pin icon.

If a polynomial model is selected, the coefficients will be displayed.

4. Click **Save As Standard Mobility Reference** to save the curve for use as a Standard Mobility Reference. In order for the curve to be saved as a Standard Mobility Reference, the curve must be a cubic polynomial and the Y variable must be Mobility. A saved Standard Mobility Reference will be available for use in subsequent analyses in the **Standard Mobility Reference** drop-down list in the **Fragment Analysis Parameters Editor** dialog box.

Editing the Dye Mobility Calibration

The Dye Mobility Calibration is a set of parameters determined with standard fragments and is used to adjust migration times or mobilities to compensate for effects of the different dyes.

WARNING **This option is provided for use by a Beckman Coulter service technician only. Changes should not be made in the dialog box unless instructed to do so by authorized Beckman Coulter personnel.**

1. Select **Analyze | Working Analysis Parameters** from the menu. The **Dye Mobility Calibration** field displays the calibration selected for the current analysis.
2. To edit the mobility calibration, click the **Edit** button.
3. Click **Advanced** in the **Fragment Analysis Parameters Editor** dialog box.
4. Click the **Edit** button next to the Dye Mobility Calibration selection.
5. Make the desired changes in the **Dye Mobility Calibration** dialog box.
6. Click **OK** to enter the changes and close the dialog box. Click **Save As** to save the dye mobility calibration as a new name.

Viewing the Dye Spectra

The dye spectra are the relative emission intensities for each of the dyes in each of the four detection channels. A new dye spectra is calculated during each sample run and the values are necessary for analysis and estimation of fragments sizes.

In some cases, the system will not be able to calculate a dye spectra from a given sample, and the analysis will fail at that point. This might happen if either of the following is true of the sample:

1. One or more of the dyes is only found in places where the signal to noise ratio is very low.
2. One or more of the dyes is only found in places where it is strongly overlapped by one or more other dyes.

If this is the case, a default set of dye spectra values will be used to perform the analysis. Viewing and saving a dye spectra stores a default set of values in the system. These values are automatically used when an analysis fails due to inability to calculate a dye spectra. Therefore, it is always good to have a default stored.

To view and save the dye spectra from a sample, perform the following steps.

1. With the desired results open, select **View | Dye Spectra**. The Dye Spectra dialog box is displayed. This dialog box displays the dye spectra for each channel.
2. Click the **Save as Default Dye Spectra** button to save the current set of values for use in future analyses. Only one set of values is stored, so clicking on the save button will replace any existing data with the current set.

A new dye spectra should be calculated and stored if any optical components have been removed, cleaned and/or replaced. This includes the optics, laser, PMT and emission collector.

Editing Fragments

Editing fragments allows you to define the fragments that are to be sized in the subsequent analysis. To activate the **Edit** mode for ignoring peaks, reinstating peaks, adding peaks or deleting added peaks, select **Tools | Edit** from the menu. To deactivate **Edit** mode, click either the **Zoom**, **Pan** or **Align** mode button.

Ignoring Peaks in the Fragment Data Pane

To ignore peaks in the Fragment Data and Fragment List, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Click on the dye color (first column) of the fragment in the fragment list corresponding to the peak to be ignored. The peak will be highlighted in the fragment data and in the data pane.
3. Click the **Ignore Peak** button or select **Edit | Ignore Peak** from the menu. The fragment will be grayed-out in the fragment list.

Reinstating Peaks in the Fragment Data Pane

To reinstate previously ignored peaks in the Fragment Data, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Highlight the grayed-out fragment in the fragment list corresponding to the peak to be reinstated. The peak will be highlighted in the fragment list and in the fragment data pane.
3. Click the **Reinstate Peak** button or select **Edit | Reinstate Peak** from the menu. The fragment in the fragment list will be un-grayed.

Adding Peaks to the Fragment List

To add peaks from the Fragment Data to the Fragment List, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Select **Edit | Add Peak** from the menu. When the cursor is placed on the fragment data pane, a small peak trace will appear under the cursor.
3. Click on the trace of the peak, as close to the apex as possible. The new peak will be added to the fragment list. This added peak will be graphically indicated by a plus sign (+) before the peak number in the fragment list. Any peak that lies between the upper and lower boundaries of the standard is available to be added. If a selected peak is not available to be added, a warning dialog box will appear.

Deleting Added Peaks in the Fragment Data Pane

To delete previously added peaks in the Fragment Data and Fragment List, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Click on the dye color (first column) of the fragment in the fragment list corresponding to the desired added peak. The peak will be highlighted in the fragment data pane and the fragment list.
3. Click the **Delete Added Peaks** icon or select **Edit | Delete Added Peaks** from the menu. The fragment corresponding to the peak will be removed from the fragment list.

Changing Fragment Dye Colors

To change the Fragment Dye Colors and default names, perform the following steps.

1. On the **Dye Colors** toolbar, click on the trace color to change the color of **D1**, **D2**, **D3** and **D4**. In the **Color** palette dialog box, select the new color and then click **OK**.
2. The default names are **D1**, **D2**, **D3** and **D4**. To change these names, launch the **Run** module. Select **File | System Preferences** and enter the desired dye names.

Hiding a Trace in the Fragment Data Pane

To hide a trace in the Fragment Data pane, click on the Dye Name in the Fragment Dye Colors toolbar. The dye name button will become raised, and the dye trace will be hidden. Up to three of the four traces can be hidden at any one time.

Viewing the Fragment List

If data has been analyzed using a Fragment Analysis Parameter Set, fragment data will be displayed with the sizes for each fragment.

To view the fragment list, select **View | Fragment List** from the menu or click on the Fragment List icon in the Sample View toolbar.

The fragment list contains all fragments identified and information pertaining to those fragments. When the list is created following the analysis of a sample, the following attributes are always displayed:

Color:	Displays the color of the dye trace for that fragment.
Std:	Displays an asterisk if the fragment is a standard peak.
Peak #:	Displays the number of the peak. (If a peak has been added, a plus sign (+) will appear in front of the peak number.)
Dye:	Displays the name of the dye for the fragment.

These items cannot be removed from the fragment list. The following attributes are optional to the fragment list and can be included/excluded by customizing the fragment list.

Customizing the Fragment List

To customize the Fragment List, perform the following steps.

1. Select **Edit | Customize Fragment List**. The **Customize Fragment List** dialog box appears.
2. Move items from the **Available** list box to **Selected** list box, and vice-versa, to add/remove them from the Fragment List. Move all items in one list box to the other by using the **All** arrow keys.
3. Move items up and down using the arrow keys to change the order of the columns in the display.

Migration Time:	Shows the average migration time for the fragment, corrected for startup ramps in separation voltage that normally occur in the method at the beginning of a run.
Standard Size:	Shows the actual size of the standard fragment in nucleotides.
Peak Height:	Displays the height of the peak.
Peak Area:	Displays the area of the peak.
Locus:	Shows the name of the locus (as entered in the Locus Tag Editor) where the fragment was identified.
Allele ID:	Displays the ID of the allele from the allele list with which the fragment is associated. For information on editing this field, see **"Editing Allele IDs"** on page 3-51.
Number of Repeats:	Displays the number of repeat units for a particular allele. (Variant alleles that contain a partial repeat are designated by the number of bases in the partial repeat. For example, a variant allele designated as 5.3 repeat units contains 5 complete repeat units and a partial repeat unit of 3 nucleotides.)
Mobility:	Displays the electrophoretic mobility of the fragment in $cm^2/V \cdot s$. This is the fragment velocity in cm/s per unit of field strength in V/cm.
Peak Width:	Displays the width of the peak.
Efficiency:	Displays the separation efficiency of the run in terms of theoretical plates.
Specific Resolution:	Displays the resolution of a pair of fragments differing in length by one nucleotide.
Estimated Size:	Displays the size of the standard and unknown fragments, estimated from the calibration curve.
Comment:	Displays system generated comments associated with a specific peak. Provides additional information about the allele identifying function of the system.

Editing Allele IDs

The Allele ID fields are the only fields in the Fragment List that can be edited. When an Allele ID has been edited, any comments related to allele identification are cleared and the word “Edited” is displayed in the comment field.

To edit an Allele ID, perform the following steps.

1. Activate the cell by double-clicking on it.
2. Enter the desired information and click out of the cell.

Showing or Hiding Standards

To show or hide the peaks corresponding to the Standard, select **Tools | Show Standards**. If the item is checked, the Standard fragments will be included in the Fragment List. If the item is unchecked the Standard fragments will be hidden in the Fragment List and the sizes for each standard peak will be hidden in the Fragment Data pane.

Showing Called Alleles Only

To show only the unknown sample peaks in the Fragment List that have been identified as alleles, select **Tools | Show Called Alleles Only** from the menu. If the item is checked, only the alleles identified from the **Allele List** dialog box will be displayed in the Fragment List and the sizes for all other peaks will be hidden in the Fragment Data pane. See ***“Specifying Allele ID Criteria”*** on page 3-44.

Data Manager Procedures

Creating a Project Folder or Database

To create a project folder or database, perform the following steps.

1. In the Data Manager window, highlight the relevant database or project folder.
2. Select **File | New** or **New Database** from the menu.
   - To create a new database, enter the name for the database in the **New Database** dialog box, check **Set as Working Database** check box (if desired) and click **OK**.
   - To create a new folder, enter the name for the folder and press **Enter**.

Deleting a Project Folder or Database

To delete a project folder or database, perform the following steps.

1. In the Data Manager window, highlight the folder or database to be deleted.
2. Select **File | Delete** from the menu.
3. In the **CEQ Data Manager** dialog box, click **Yes** to delete the folder or database.

You can not delete the working database.

CAUTION **When a folder is deleted, all of the data contained within that folder will be deleted. You will be warned by the system.**

Renaming a Project Folder or Database

To rename an existing folder, perform the following steps.

1. In the Data Manager window, highlight the project folder or database.
2. Select **File | Rename** from the menu.
3. Enter the new name for the database and press the **Enter** key.

Compacting a Database

To increase the storage efficiency of the database data on the hard drive, perform the following steps.

1. In the Data Manager window, highlight the database to be compacted.
2. Select **Tools | Compact** from the menu.
3. Select **OK** from the **CEQ Data Manager** dialog box to clear the dialog box. The database will be compacted.

Repairing a Database

To repair the database, perform the following steps.

1. In the Data Manager window, highlight the database to be repaired.
2. Select **Tools | Repair** from the menu.
3. Select **OK** from the **CEQ Data Manager** dialog box to clear the dialog box. The database will be repaired.

Exporting Database Items

To export database items to other folders, perform the following steps.

1. In the Data Manager window, select the appropriate database and project, then highlight the filename in the right-hand window listbox.
2. Select **File | Export** from the menu.
3. In the **Export** dialog box, select the desired export format from the **Save as type** drop-down menu), select the file(s) to export under the **Selected Files** listbox, locate the target folder, and then click **Export**.

Importing Database Items

To import items into the database, perform the following steps.

1. In the Data Manager window, select the project where the imported file will reside.
2. Select **File | Import** from the menu.
3. From the **Import** dialog box, locate the folder where the file resides, select the import format from the **Files of type** drop-down menu, highlight the filename, and then click **Import**.

Generating a Sample Run History

To generate a sample run history, perform the following steps.

1. Select **View | Sample Run History** from the menu.
2. In the **Sample Run History** dialog box:
   a. Select a **Project** name from the drop-down menu.
   b. Select **Enable** and enter a filtering start and end date.
   c. Click the **Compute** button.
   d. View the total number of runs during the specified dates in the **Total Sum of Samples Run** field.
   e. Print a report of all samples run during the specified dates by selecting the **Print** button.

Customizing the Master Database

When you create a new database, the software uses a master database as the default starting database. To customize the master database so it contains specific items that will be part of *every* new database, perform the following procedures.

You may modify the master database as many times and as often as needed.

If you delete any items during this procedure, you should compact the database in order to reclaim any lost space in the database.

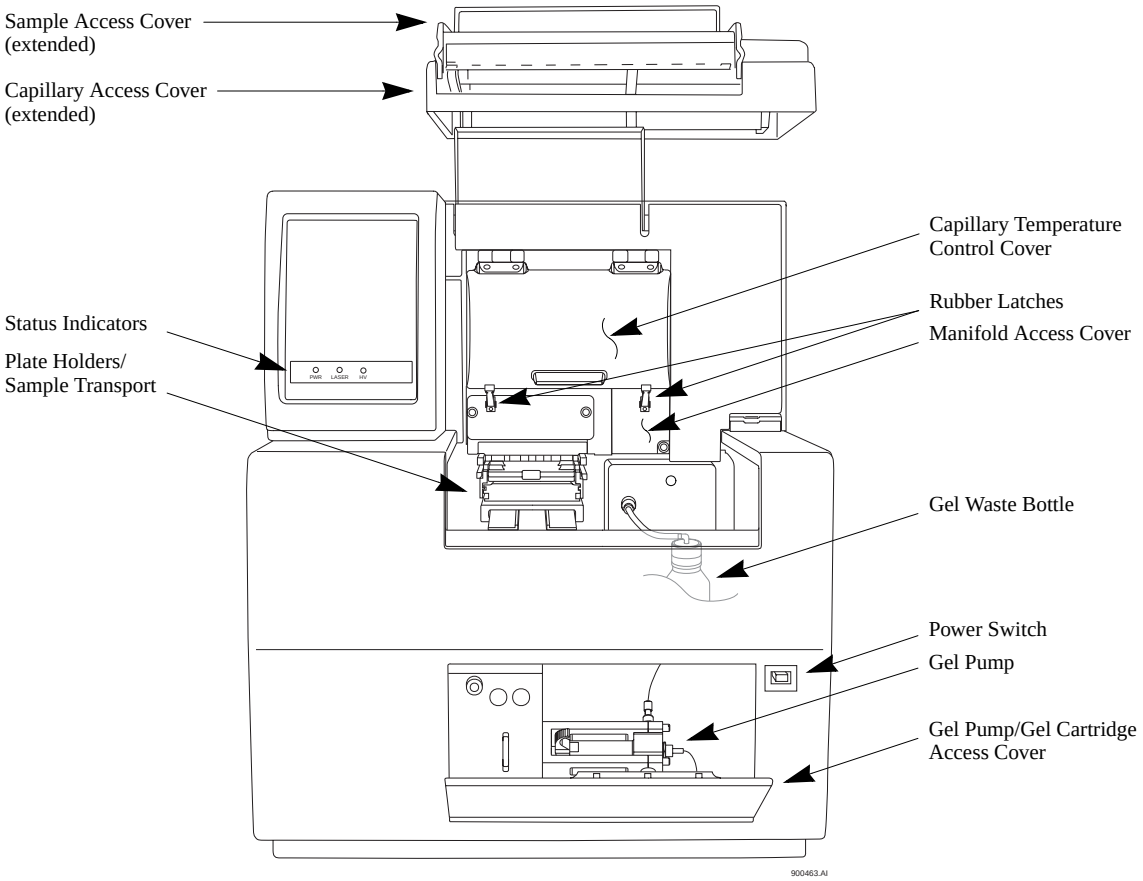
1. Create a new database by clicking on **File | New Database.**
2. Enter a name for the new database and select **Set As Working Database** in the **New Database** dialog box.
3. Modify any of the following database items: Fragment Analysis Parameters, Locus Tags, Methods, Sample Plates, Sequence Analysis Parameters, and Standards.
4. After modifying the database, specify another database as the working database.
5. Exit the CEQ 2000 application.
6. Access Windows Explorer.
7. In the CEQ 2000 software folder, open the folder named Dbase.
8. Delete the file named CEq-Master.xdb.
9. Highlight the database you just created / modified. Rename it CEq-Master.xdb. This master will now be used as the default starting database whenever you create a new database.

Direct Control and Replenishment Procedures

The following procedures apply to the Direct Control functions of the Run module. Direct Control functions can be accessed via the **Direct Control** menu, the **Replenish** menu and the **Hot Areas** of the Direct Control window.

Use Figure 72, User Accessible Hardware Components, to locate hardware components referenced in this section.

Figure 72: User Accessible Hardware Components



Accessing the Direct Control Window

From the Shortcut Bar, click on the **Run** icon. Verify that the Run window is displayed and then click on the **Direct Control** tab.

The following procedures can initiated from the hotspots in the Direct Control window, by right-clicking anywhere in the Direct Control window to access the Direct Control / Replenish menu or by clicking on Direct Control from the main menu.

Direct Control Procedures

Replacing the Wetting Tray

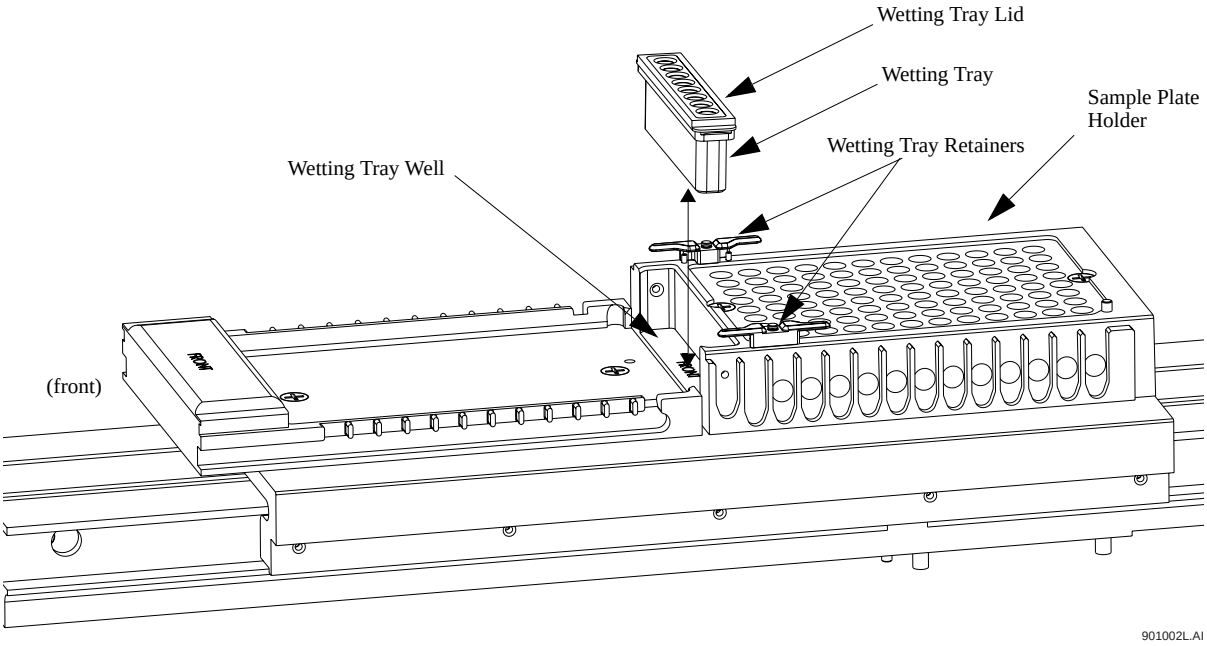
Removing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the menu.
2. Open the Sample Access Cover (Figure 72) and lift to the vertical locking position.
3. Remove the sample plate and set aside.
4. Rotate the Wetting Tray Retainers outwards to release the Wetting Tray.
5. Lift the Wetting Tray vertically.

Installing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the menu.
2. Open the Sample Access Cover (Figure 72) and lift to the vertical locking position.
3. Insert the Wetting Tray into the receptacle between the Sample and Buffer plates (Figure 73) and then gently press it down into the well.
4. Rotate the Wetting Tray Retainers inwards to lock the Wetting Tray in place.
5. Install the Sample Plate.

Figure 73: Replacing the Wetting Tray



6. Close the Sample Access Cover and then click the **Done** button in the **Replace Wetting Tray** dialog box (Figure 74).

Figure 74: Replace Wetting Tray Dialog

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Loading the Sample Plate or Buffer Plate

1. Select **Direct Control | Unload Plates** from the menu.

Figure 75: Unload Plates Dialog

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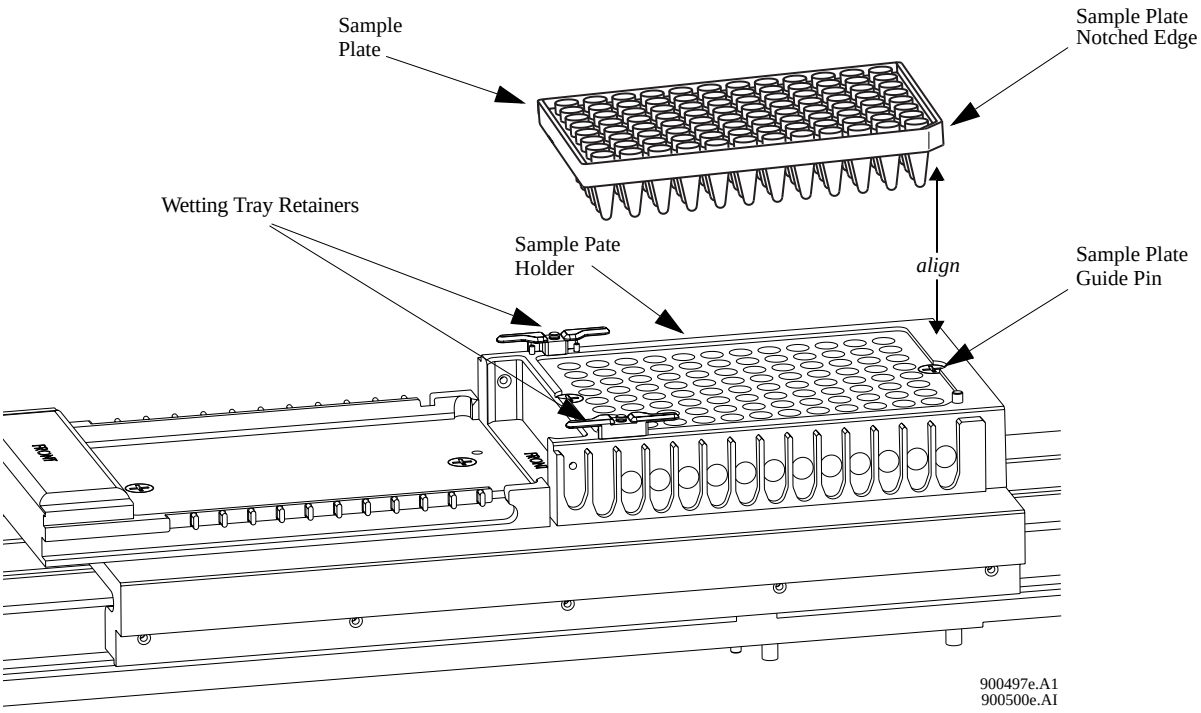
2. In the **Unload Plates** dialog box, select the **Sample Plate**, **Buffer Plate** or **Wetting Tray** radio button. This is where the capillaries will be immersed after the load procedure is performed.
3. If **Sample Plate** or **Buffer Plate** was selected, use the **Position Set** spin control to identify the position of sample or buffer and then click **Unload**. If **Wetting Tray** was selected, click **Unload**.
4. Open the Sample Access Cover (Figure 72) and lift to the vertical locking position.
   - If **Sample Plate** was selected, skip to "***Loading the Sample Plate***."
   - If **Buffer Plate** was selected, skip to "***Loading the Buffer Plate and Evaporation Cover***."

Loading the Sample Plate

1. Make sure the Wetting Station is installed or perform the procedure "***Installing the Wetting Tray***" on page 4-56, and then return here.
2. Align the Sample Plate Guide Pin with the notched edge of the sample plate and gently lower the plate into position (Figure 76).

Figure 76: Loading the Sample Plate



3. When finished positioning the plate, close the Sample Access Cover and then click the **Load** button of the **Capillaries Exposed** dialog box (Figure 77).

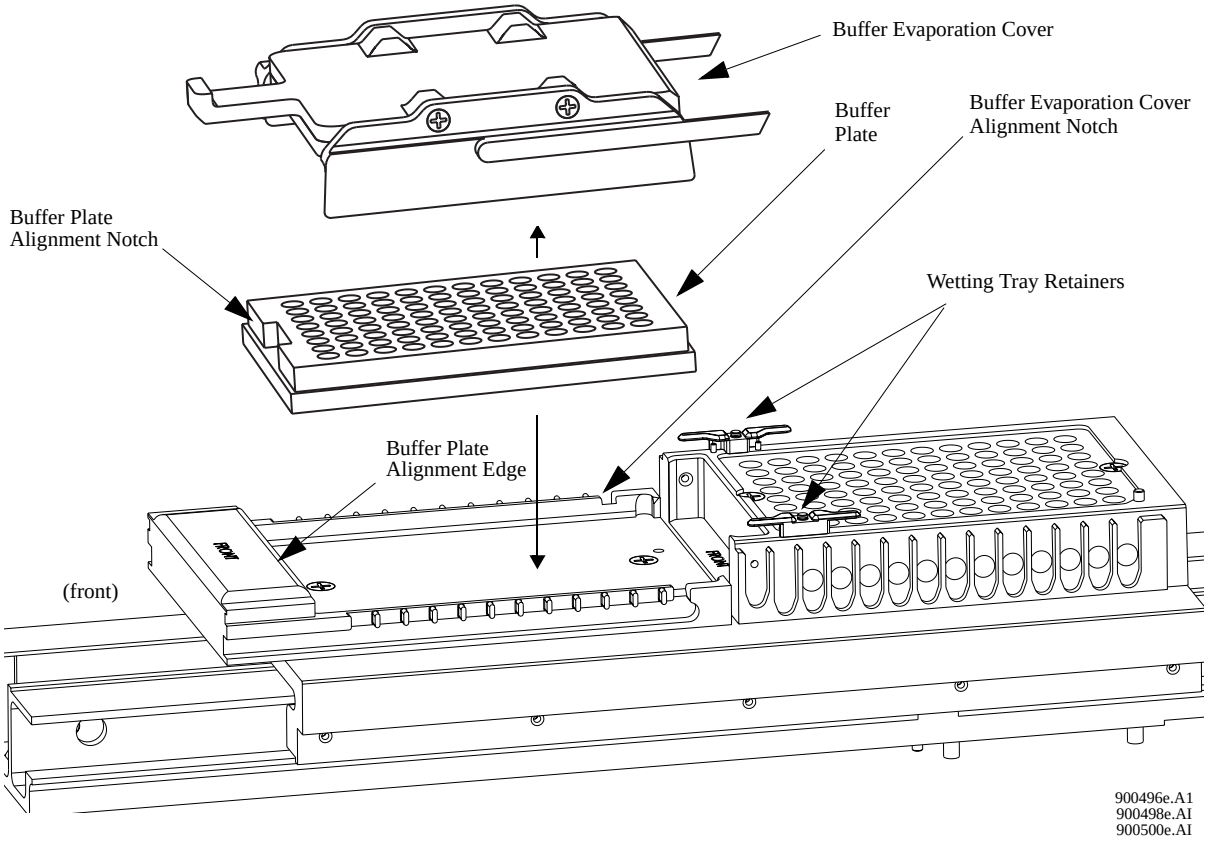
CAUTION **The separation gel within the capillaries will dry out if the capillaries are left exposed to the air for more than 15 minutes.**

Figure 77: Capillaries Exposed Dialog

Loading the Buffer Plate and Evaporation Cover

1. At the front of the Sample Transport (Figure 78), set the Buffer Plate Alignment Notch underneath the alignment edge of the transport.
2. Gently push the Buffer Plate towards the front of the instrument and then set the plate into the transport.
3. At the rear of the Buffer Plate Holder, align the Buffer Evaporation Cover Guide Pin with the Buffer Evaporation Cover Alignment Notch and then gently lower the cover over the Buffer Plate.

Figure 78: Loading the Buffer Plate and Buffer Evaporation Cover



4. When finished positioning the plate, close the Sample Access Cover and then click the **Load** button of the **Capillaries Exposed** dialog box (Figure 77).

Specifying Plate Positions

1. Select **Direct Control | Plate Position** from the menu.
2. In the **Plate Position** dialog box, select the **Sample Plate** or **Buffer Plate** sample set or **Wetting Tray** set to identify the appropriate position where the capillaries will be immersed.

Specifying Capillary Temperature

1. Select **Direct Control | Capillary Temperature** from the menu.
2. In the **Capillary Temperature** dialog box:
   a. Enter a capillary holding temperature value in degrees centigrade.
   b. Select **Wait for temperature to be reached** and then click **Start**.

Denaturing a Sample

1. Select **Direct Control | Denature** from the menu.
2. In the **Denature Samples** dialog box:
   a. Enter a time duration in seconds.
   b. Identify the position of the sample using the **Sample Set** spin controls and then select **Denature**.

Injecting a Sample

1. Select **Direct Control | Inject** from the menu.
2. In the **Inject** dialog box:
   a. Enter a value for the voltage in kV.
   b. Enter a time duration in seconds.
   c. Identify the position of the sample using the **Sample Set** spin controls and then select **Inject**.

Performing a Separation

1. Select **Direct Control | Separate** from the menu.
2. In the **Separate** dialog box:
   a. Enter a value for the voltage in kV.
   b. Enter a time duration in minutes.
   c. Identify the position of the buffer using the **Buffer Set** spin controls and then select **Separate**.

Replenishing the Capillaries with Gel

1. Select **Direct Control | Gel Capillary Fill** from the menu.
2. From the **Gel Capillary Fill** dialog box, select the **Buffer Plate** or **Wetting Tray** radio button to identify the position where waste will be expelled from the capillaries.
   a. If **Buffer Plate** was selected, use the **Buffer Plate** spin controls to identify the waste position in the buffer plate and then select **Fill**.
   b. If **Wetting Tray** was selected, click the **Fill** button.

Purging the Manifold

1. Select **Direct Control | Manifold Purge** from the menu.
2. In the **Manifold Purge** dialog box:
   a. Enter a volume in milliliters (mL).
   b. Enter the number of cycles and then select **Purge**.

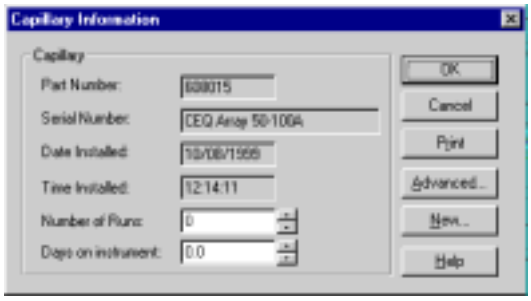
Replenishment Procedures

Viewing or Changing Capillary Information

The capillary array used to produce raw data for sequence analysis differs from the array used to produce raw data for fragment analysis. For this reason, it is important to enter the correct information in the dialog boxes pertaining to the capillary array.

1. Select **Replenish | Capillary Information** from the Run module menu bar.
2. Use the **Capillary Information** dialog box (Figure 79) to view or change the following information:
   a. View the part number of the installed capillary array.
   b. View the serial number used to track the capillary array along with the samples that used the array.
   c. The system automatically updates the date and time installed.
   d. Change the number of runs imparted on the capillary array since it was installed using the spin control, if you wish to change the system's alert mechanism.
   e. Change the number of days the capillary array has been on the instrument using the spin control, if you wish to change the system's alert mechanism.
   f. Click **Advanced** to view additional information specific to the installed array.
   g. Click **OK** to enter the information and close the dialog box.
3. When finished, select **OK** to close the window(s).

Figure 79: Capillary Information Dialog



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Viewing or Changing Gel/Buffer Information

1. Select **Replenish | Gel Cartridge/Buffer Information** from the Run menu.
2. Use the **Gel Cartridge/Buffer Information** dialog box (Figure 80) to view and/or change the following information:
   a. View the gel part number or select a new buffer part number.
      To enter a new gel or buffer part number, click the **New** button. In the **New Gel Cartridge/Buffer** dialog box (Figure 81), click in the appropriate radio button and enter the part number in the active field. The new part number will appear in the appropriate **Part Number** field or drop-down menu.
   b. The system automatically updates the date and time installed.
   c. Use the spin control to change the **Hours on Instrument** if you wish to change the system's alert mechanism.
3. When finished, select **OK** to close the window.

Figure 80: Capillary Information Dialog

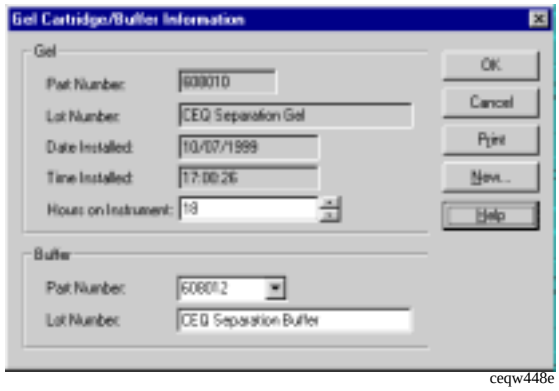

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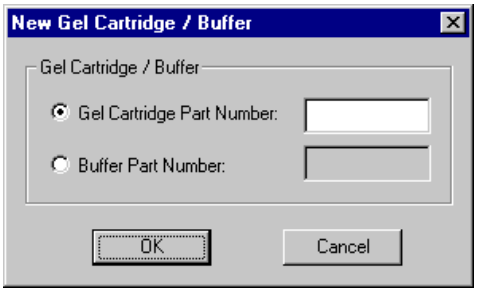
Figure 81: New Gel Cartridge/Buffer Dialog



Removing and Replacing the Capillary Array

CAUTION **Care must be exercised when installing/removing the capillary array to prevent damage and maintain low background signals. Clean all gel residues thoroughly.**

This procedure assumes that an expended capillary array is being replaced with a new capillary array.

1. Select **Replenish | Release Capillary Array** from the Run menu. After the system prepares for release of the capillary array, the **Remove Capillary Array** dialog box (Figure 82) is displayed.

Figure 82: Remove Capillary Array Dialog

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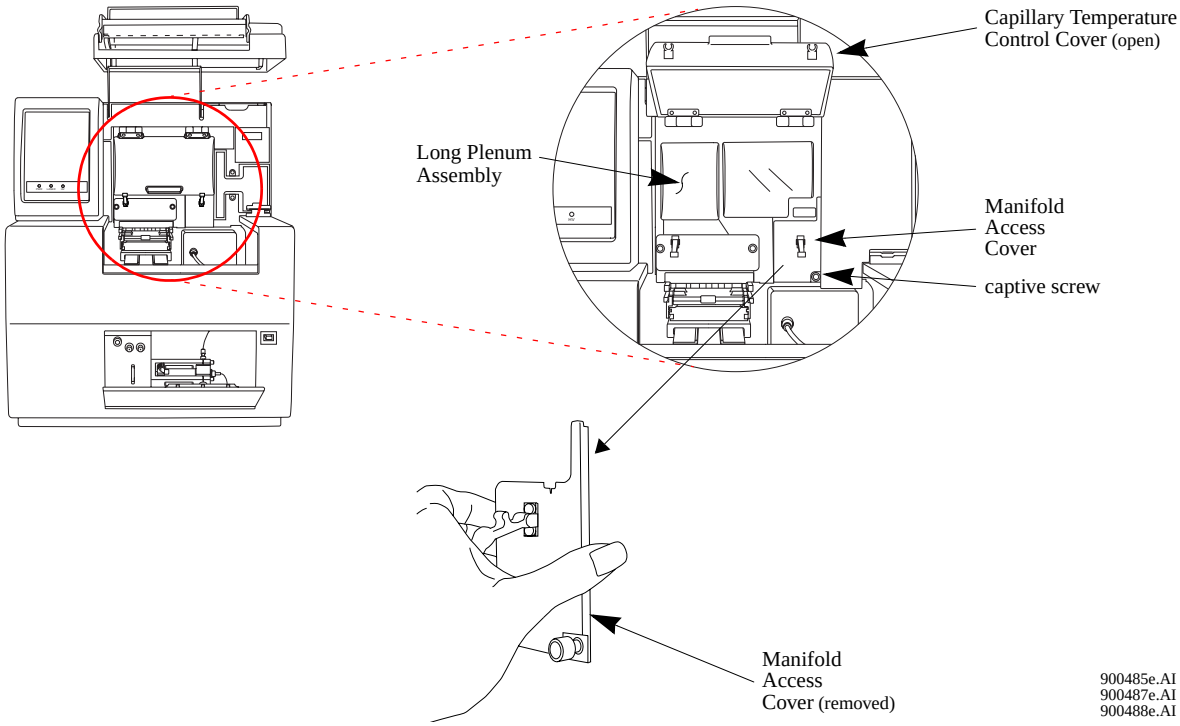
2. Open the Sample Access Cover (Figure 72) and lift to the vertical locking position.
3. Open the Capillary Access Cover and lift to the vertical locking position.
4. Unlatch the two rubber latches holding the Capillary Temperature Control Cover and lift to the vertical locking position.
5. Loosen the Manifold Access Cover captive screw (Figure 83), remove the cover and set it aside.

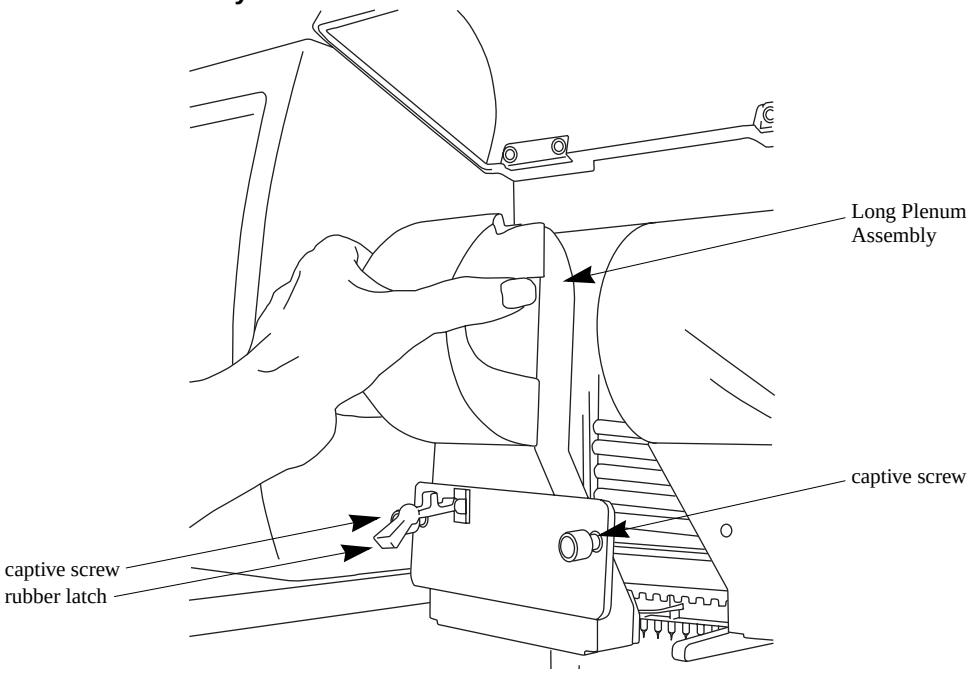
Figure 83: Manifold Access Cover



6. Loosen the two Plenum Assembly captive screws (Figure 84), pull the Plenum Assembly straight back and away from the instrument and set it aside.

CAUTION **Slowly remove the Plenum Assembly as the electrode block may disengage from its mounting posts and become damaged.**

Figure 84: Plenum Assembly Removal

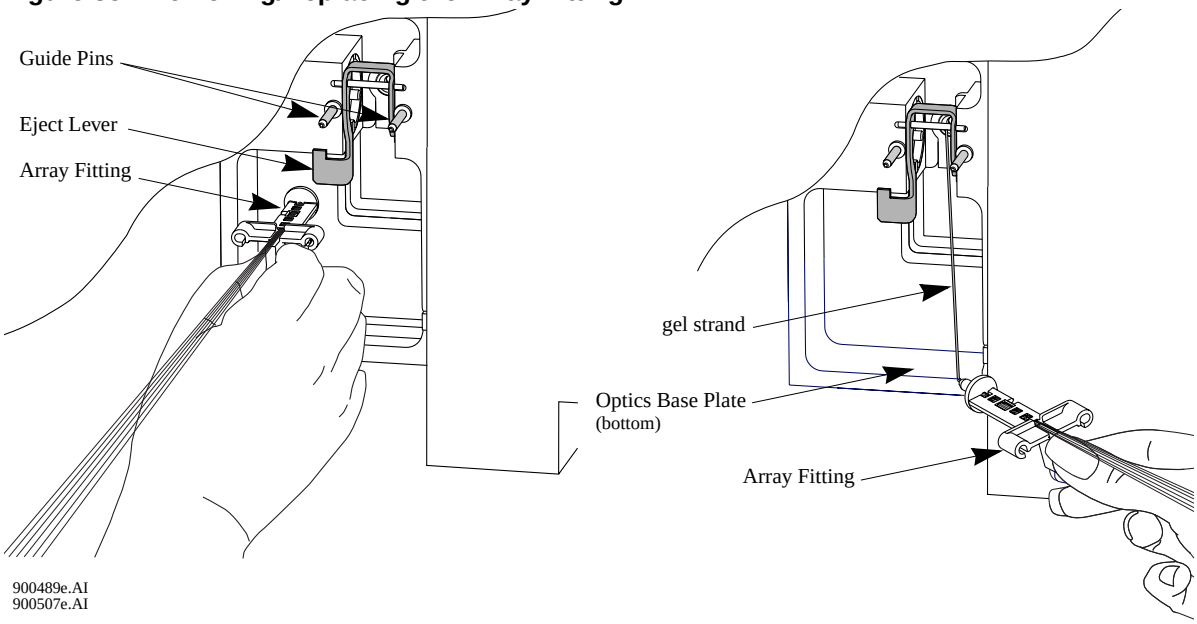


7. Lift the Eject Lever (Figure 85) to release the Array Fitting.

8. Grasp the Array Fitting tab (Figure 85) and then:
   a. Pull the fitting approximately one inch out of the manifold.
   b. Touch the tip of the fitting to the bottom of the Optics Base Plate.
   c. Hold and wait five seconds for the gel strand to dry.
   d. Pull the fitting away from the instrument.
   e. Wipe gel strands off of the instrument using a damp tissue.

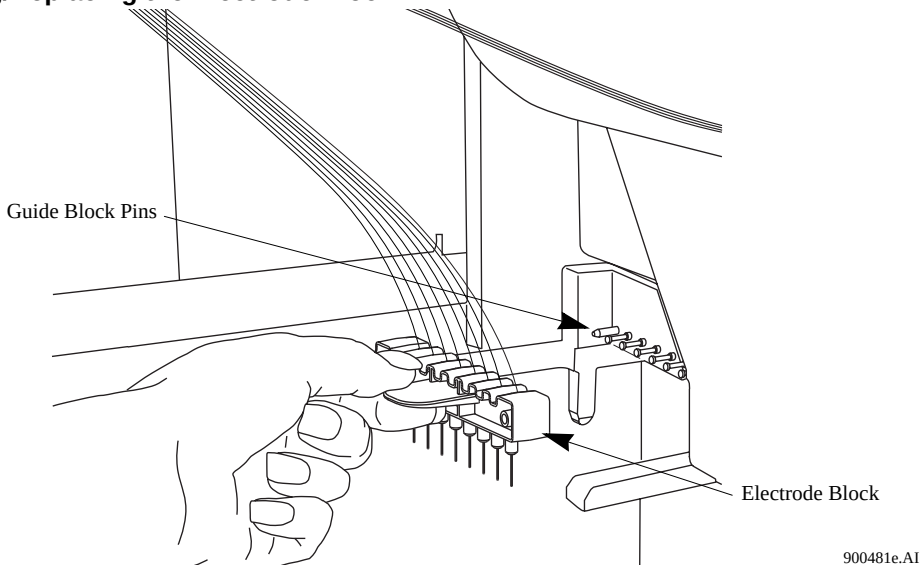
Note the number of runs and days on the instrument for this capillary array for reference. If this capillary array is to be re-installed, this information is necessary.

Figure 85: Removing/Replacing the Array Fitting



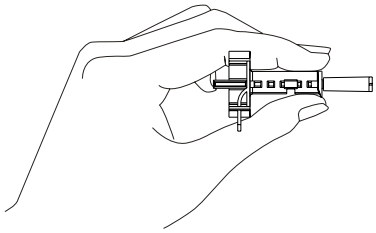
9. Grasp the Electrode Block tab (Figure 86) with your left hand and pull the block out and away from the instrument.

Figure 86: Removing/Replacing the Electrode Block



10. From the **Remove Capillary Array** dialog box (Figure 82), select the **Replace Capillary Array** radio button and click **OK**.

CAUTION **Always grip the capillary array fitting near the end during removal or installation of the tip cap to prevent flexing and possible breakage of the capillary array.**



11. While holding the Array Fitting tab (of the new capillary array), align the Array Fitting (Figure 85) with the manifold opening and guide pins. Push the fitting into the manifold until it is completely seated against the bases of the Guide Pins. Make sure that the fitting tab is placed downward when inserting the capillary array into its slot.
12. While holding the Electrode Block tab, align the Electrode Block (Figure 86) with the guide block pins and gently push it in until resistance is met. (The resistance is from the spring-loaded contacts.)

Figure 87: Long Plenum Assembly - Front View

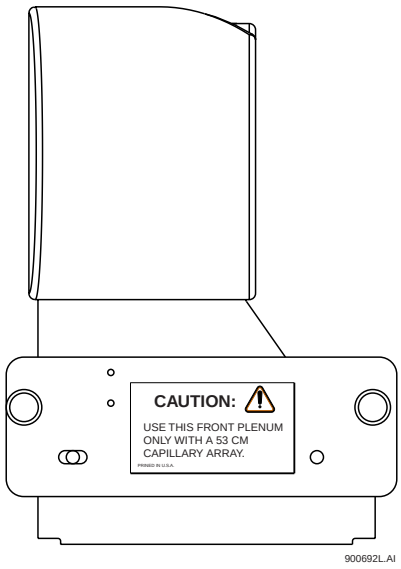
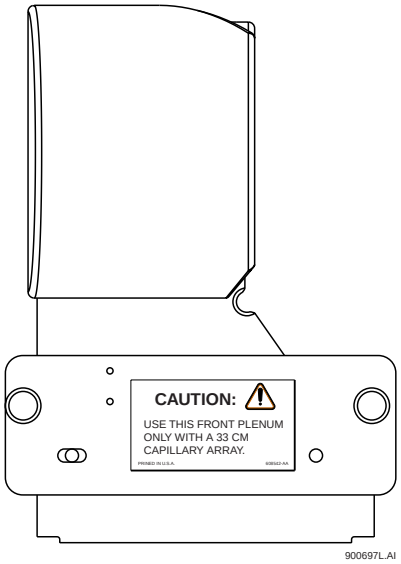


Figure 88: Short Plenum Assembly - Front View



13. Make sure that the capillary array is routed through the proper path.
    a. When installing the 53 centimeter capillary array, route the capillaries over the top right-hand side of the Long Plenum Assembly (Figure 87) and into the guide block pins, resting them in the groove in the helical enclosure (Figure 89).
    b. When installing the 33 centimeter capillary array, carefully route the capillaries through the hole in the Short Plenum Assembly (Figure 88) and straight across into the guide block pins (Figure 90).

Figure 89: Long Capillary Array Routing

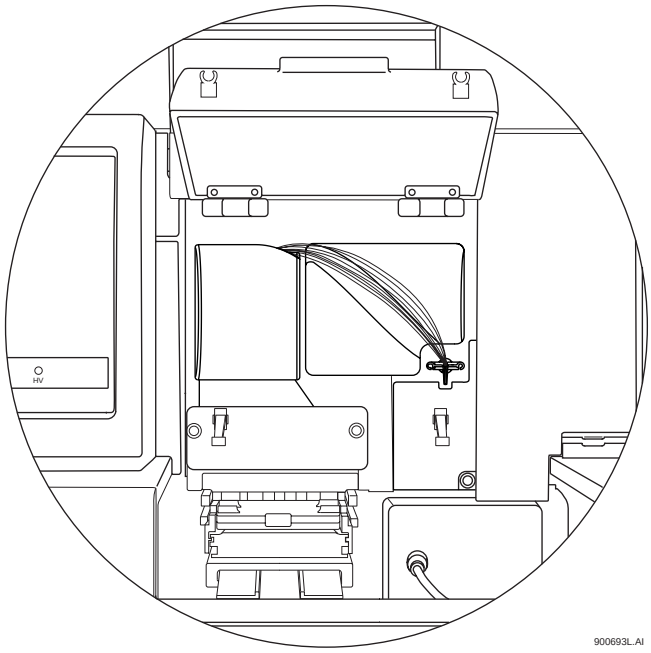
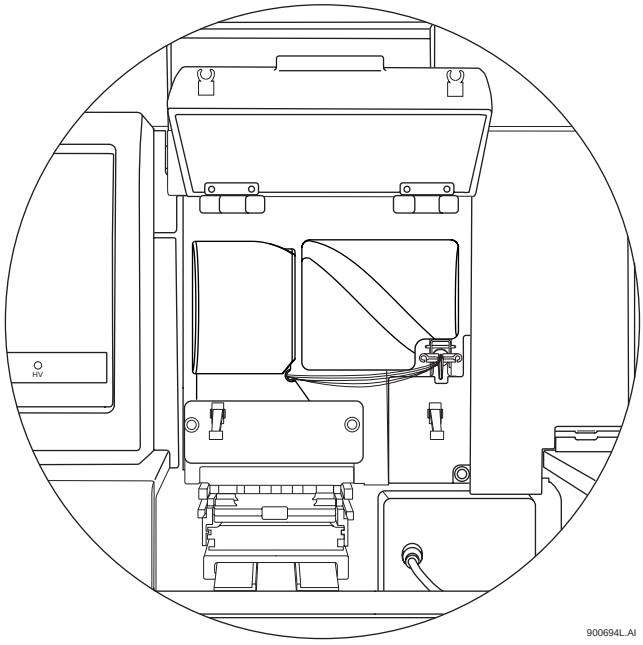


Figure 90: Short Capillary Array Routing



When reinstalling the Short Plenum Assembly, gently gather the capillaries between your thumb and forefinger to make sure they pass through the hole in the Short Plenum Assembly without any constriction.

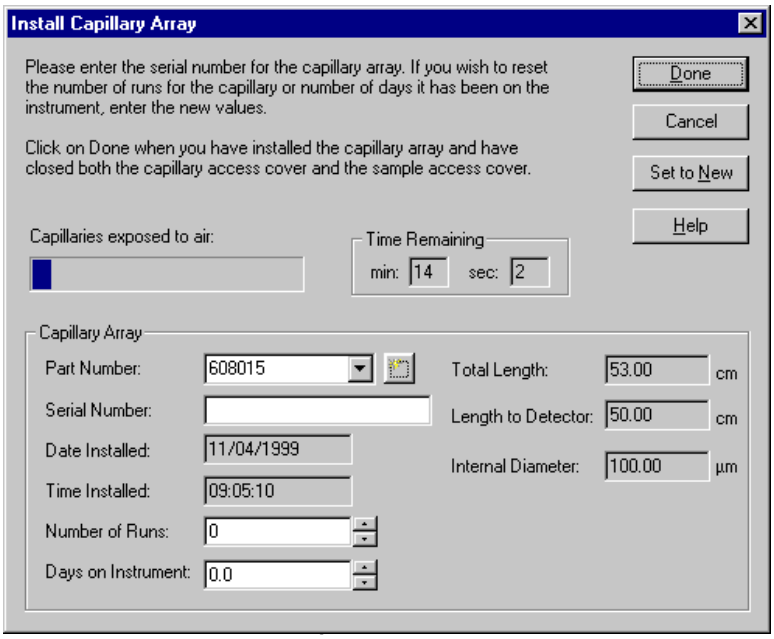
14. Replace the Plenum Assembly (Long or Short) and tighten the two captive screws (Figure 84).
15. Replace the Manifold Access Cover and tighten the captive screw (Figure 83).
16. Lower the Capillary Temperature Control Cover and secure the two rubber latches.
17. Lower the Capillary Access Cover and Sample Access Cover to their locking positions
18. If you are installing a new capillary array, in the **Install Capillary Array** dialog box (Figure 91), select the correct part number, enter the serial number, click the **Set to New** button then on **Done**. The number of runs and days on instrument will revert to "0."

    If you are installing the previous capillary array, do not change the serial number, number of runs, or the number of days on the instrument as they will be correct.

    If you are installing a capillary array that was previously used, but not the last capillary array on the instrument, enter its part number (if applicable) serial number, and adjust the number of runs and the previous cumulative days on the instrument. Then click **Done**.

    Note the number of runs and days on the instrument for this capillary array for reference.

Figure 91: Install Capillary Array Dialog



Conditioning the Capillaries

A new capillary array must be conditioned before use. Perform the following procedure every time you install a new array in the system.

1. In the Sample Setup module, set up one row and select the "Condition" method.
2. Fill the corresponding wells in the plate with formamide.
3. Disable **Automatic Analysis** on the Analysis tab.
4. Select **Run | Start** from the menu.

If you are running an older version of CEQ 2000 DNA Analysis Software, you can import the method as a CEQ.* file.

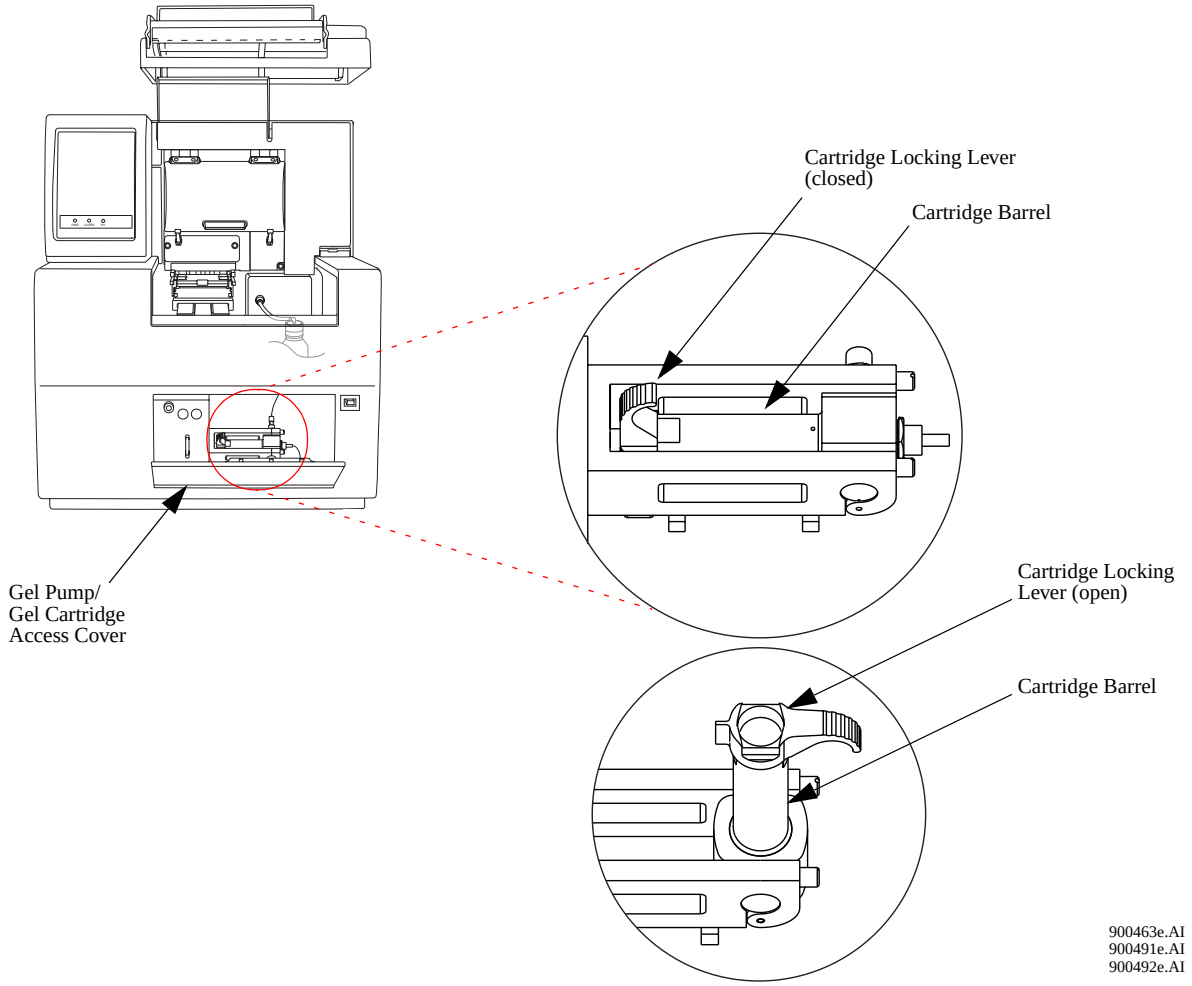
Removing and Replacing a Gel Cartridge/Gel Pump Plug

CAUTION **Care must be exercised when installing/removing a gel cartridge due to the viscosity of the gel mixture. Do not allow gel to remain on the instrument.**

This procedure assumes that an expended gel cartridge is being replaced with a fresh gel cartridge or that a used gel cartridge is being removed (for storage purposes) and being replaced with the gel pump plug.

1. Select **Replenish | Release Gel Cartridge** from the Run menu. When the system is ready to release the gel cartridge, the **Release Gel Cartridge** dialog box will be displayed. Wait until the lead screw is completely disengaged.
2. Open the Gel Pump/Gel Cartridge Access Cover by gently pushing in on the top of the cover. (The cover is spring-loaded and will pop open.)
3. Pull on the Cartridge Locking Lever. (The barrel will swing outwards to approximately a 90° angle from its locked position.)

Figure 92: Removing/Replacing the Gel Cartridge

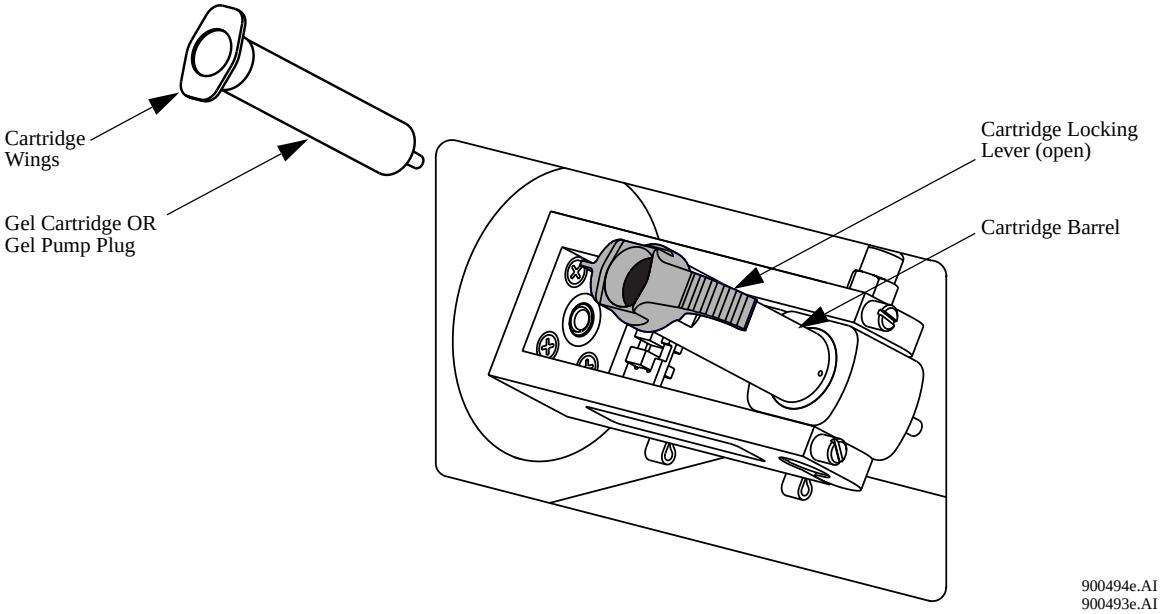


4. Grasp the wings of the gel cartridge/gel pump plug and pull it out of the barrel.

> ***Always note the lot number and the hours on the instrument for a gel cartridge if you are planning to use it for more than one session.***

5. If necessary, use a tissue to wipe gel strands off of the instrument.

Figure 93: Removing/Replacing the Gel Cartridge - Continued



6. Remove any air pockets from the gel cartridge by grasping the cartridge wings between your first two fingers, and depressing the plunger with your thumb until a small amount of gel is pushed out of the cartridge tip.

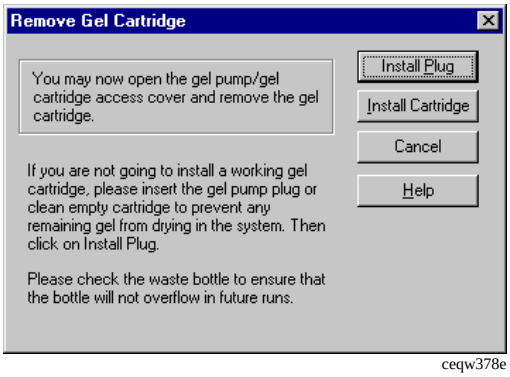
   If there are bubbles present in the gel, do not install the cartridge. Repeat step 6 above before installing this cartridge.

7. Insert the new cartridge or the gel pump plug into the barrel and lock it into position by aligning the cartridge wings with the cartridge holder and pushing in.
8. Push the cartridge locking lever towards the back of the instrument (approximately a 90° angle) into its locked position.
9. Close the Gel Pump/Gel Cartridge Access Cover.
10. In the **Remove Gel Cartridge** dialog box (Figure 94):
    - Click **Install Cartridge** to indicate that a gel cartridge was installed.

    OR

    - Click **Install Plug** to indicate that the gel pump plug (or empty cartridge) was installed.

Figure 94: Remove Gel Cartridge Dialog



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11. If **Not Installed** was selected in step 10, proceed to step 12.

    If **Install Cartridge** was selected in step 10, use the **Install Gel Cartridge** dialog box (Figure 95) to enter the following information:

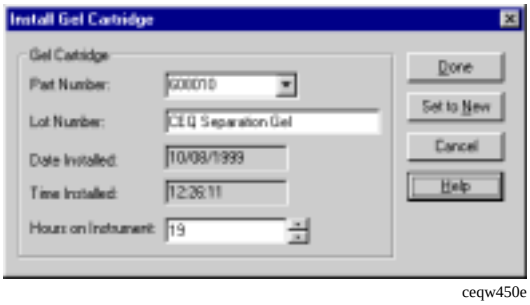
    a. If you are installing a new gel cartridge, click the **Set to New** button, select the part number from the drop-down menu, enter the lot number, and then click **OK.** The system will automatically update the date and time installed and set the **Hours on Instrument** to "0."
    b. If you are installing the previous gel cartridge, do not change the lot number, or the hours on the instrument, as they will be correct.
    c. If you are installing a previously used gel cartridge, but it was not the last one on the instrument, enter its part number and/or lot number, and adjust the hours to properly reflect the hours this cartridge has been on the instrument.

    Always note the lot number and the hours on the instrument for a gel cartridge if you are planning to use it for more than one session.

    The lot number is an alphanumeric text box for your own identification purposes.

    CAUTION ***If the gel cartridge has been on the instrument for more than 72 hours, it is likely that the gel will produce undesirable results.***

Figure 95: Install Gel Cartridge Dialog



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12. Dispose of the used tissue and spent gel cartridge in accordance with the procedure, "***Disposal of the Gel Cartridge***" on page 4-11.

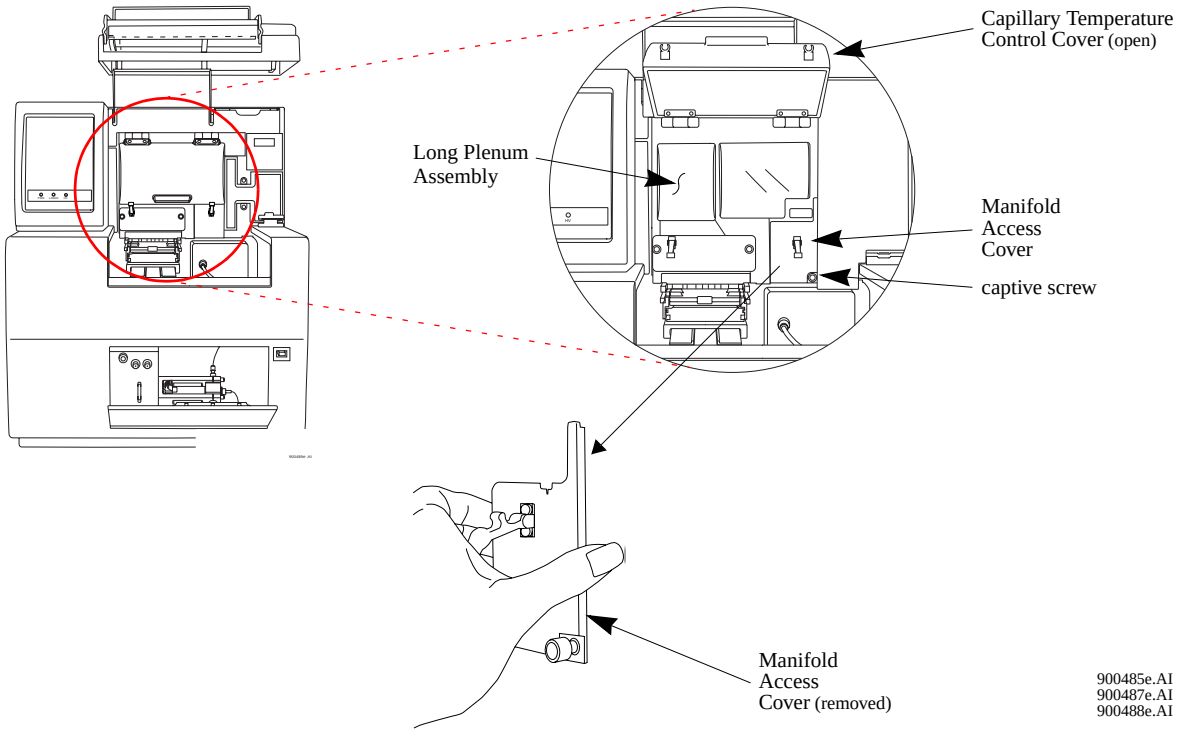
Removing the Manifold Plug

The Manifold Plug is installed in the CEQ instrument during shipping and when the instrument is not is use to prevent drying of gel.

This procedure assumes that the Manifold Plug is being removed in preparation for Capillary Array installation.

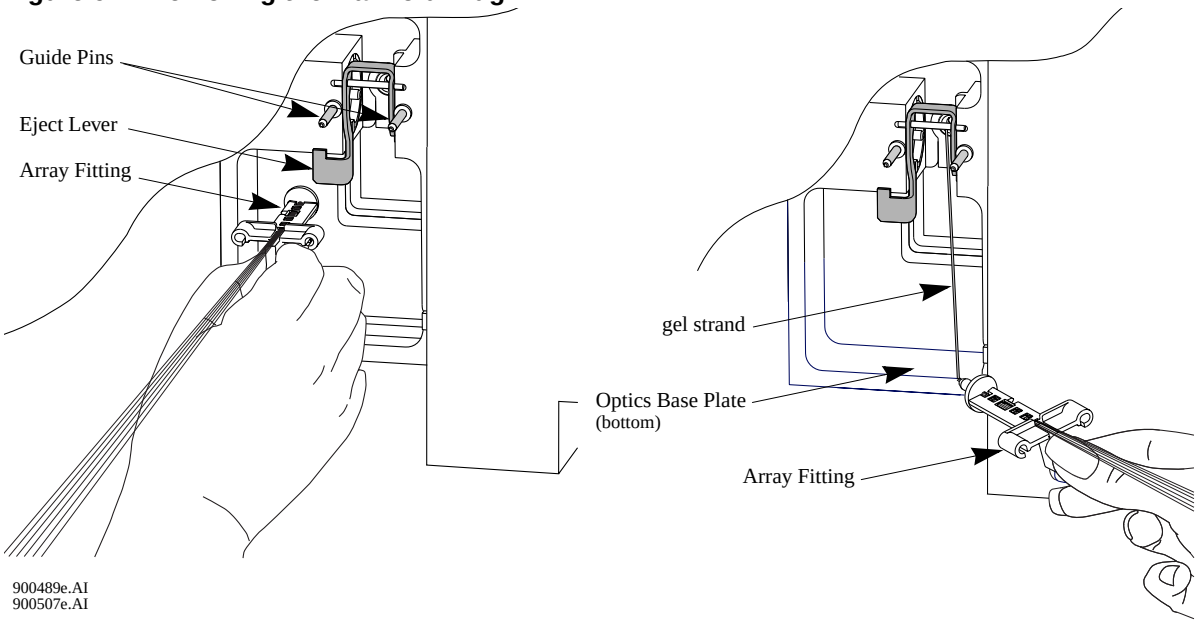
1. Select **Replenish | Release Capillary Array** from the Run menu.
2. Open the Sample Access Cover (Figure 72) and lift to the vertical locking position.
3. Open the Capillary Access Cover and lift to the vertical locking position.
4. Unlatch the two rubber latches holding the Capillary Temperature Control Cover and lift to the vertical locking position (Figure 96).
5. Loosen the Manifold Access Cover captive screw, remove the cover and set it aside.

Figure 96: Manifold Access Cover



6. Lift the Eject Lever to release the Manifold Plug.
7. Grasp the Manifold Plug tab (Figure 97) and then:
   a. Pull the plug approximately one inch out of the manifold.
   b. Touch the tip of the plug to the bottom of the Optics Base Plate.
   c. Hold and wait five seconds for the gel strand to dry.
   d. Pull the plug out and set it aside for future use.
   e. Wipe gel strands off of the instrument using a damp tissue.

Figure 97: Removing the Manifold Plug



8. Select **OK** from the **Remove Manifold Plug** dialog box (Figure 98).

Figure 98: Remove Manifold Plug Dialog

9. To install the capillary array, see "***Removing and Replacing the Capillary Array***" on page 3-63.

Diagnostic Procedures

Re-Initializing the System

To re-initialize the system, select **Run | Reset** from the Run menu.

Homing the Plates and/or Gel Pump

To re-establish the position of the plates and/or gel pump, perform the following steps.

1. Select **Run | Diagnostics** from the Run menu.
2. From the **Diagnostics** dialog box, select the **Home Plate** and/or **Gel Pump** check box and click **OK**.
3. Monitor the **Tests** dialog box and verify that the final result(s) is **Test Passed** and then click the **Close** button.

Viewing PC Settings

To view the computer settings, perform the following steps.

1. Select **Run | Diagnostics** from the Run menu.
2. From the **Diagnostics** dialog box, select the **PC Settings** button.
3. View the settings in the **PC Communication Settings** dialog box and then exit the dialog box.

Viewing Instrument Status

To view the instrument status, perform the following steps.

1. Select **Run | Diagnostics** from the Run menu.
2. From the **Diagnostics** dialog box, select the **Status** button.
3. View and/or change the settings in the **CEQ Monitor** dialog box and then select **OK**.

Viewing Optical Scan Data

To view the optical scan data in either the Sequence or Fragment Analysis module, perform the following steps.

1. Select **File | Open** from the desired Analysis module menu.
2. In the **Open** dialog box, select the **Optical Scan Data** tab, highlight the desired sample name and then select **OK**.
3. Verify that the **Optical Scan** window is displayed.

Performing an Optical Alignment

To align the lasers with the detection windows of the eight capillaries, perform the following steps.

1. Select **Direct Control | Optical Alignment** from the Run menu.
2. To save the alignment data:
   a. Select the **Autosave** check box.
   b. Enter a name in the **Name** field.
   c. Select a **Project** from the drop-down menu.
   d. Select **Align**.

Monitoring the Baseline

You must perform the optical alignment procedures prior to monitoring the baseline.

To monitor the baseline, perform the following steps.

1. Select **Run | Monitor Baseline** from the Run menu.
2. From the **Monitor Baseline** dialog box:
   a. Select the **Enable Monitor Baseline** check box.
   b. Select the **Autosave** check box (to save monitor baseline data).
   c. Enter a name in the **Name** field.
   d. Select a **Project** from the drop-down menu and then select **OK**.
3. To view the baseline trace:
   a. Access the desired Analysis module.
   b. Select **File | Open** from the menu.
   c. Click on the **Sample Data** tab and select the desired baseline data (the baseline data is displayed).

Chapter Four 4

Routine Maintenance

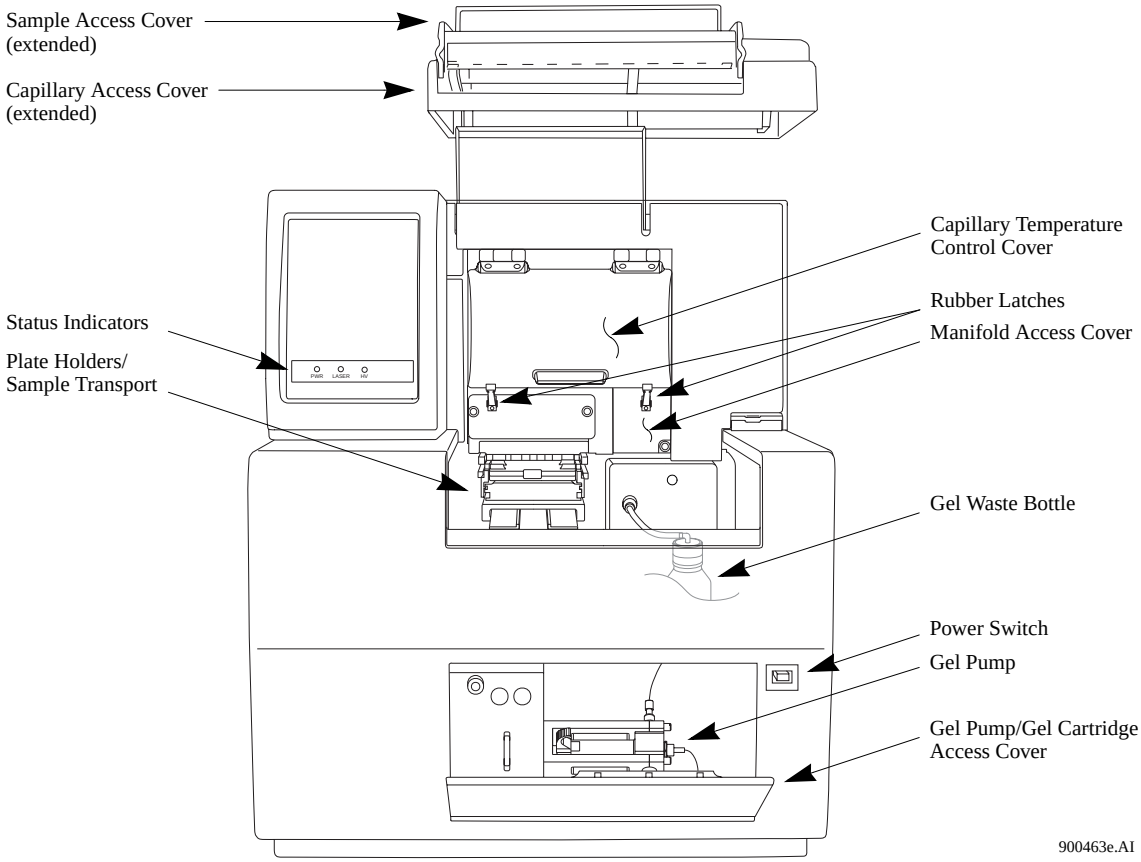
Chapter Overview

This section provides routine maintenance and biological waste disposal procedures. It also provides a list of the consumable materials used in the system.

Routine Maintenance

Use Figure 99, User Accessible Hardware Components, to locate hardware components referenced in this chapter.

Figure 99: User Accessible Hardware Components



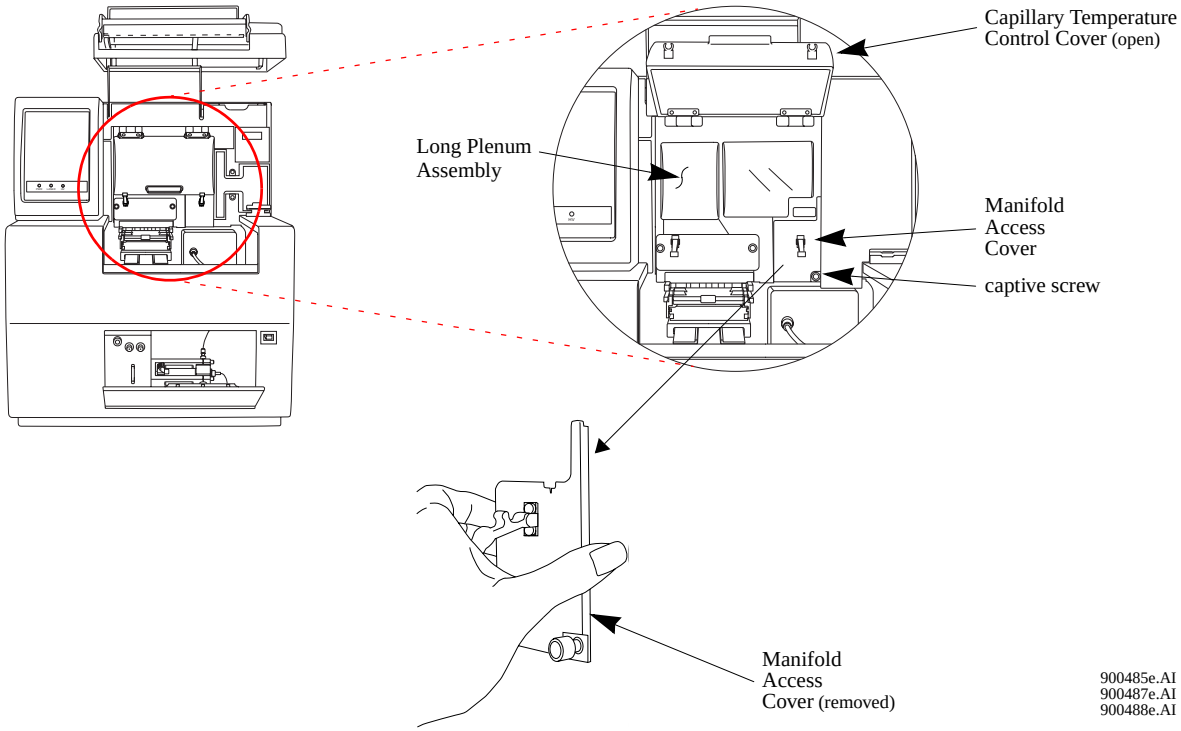
Cleaning the Capillary Array

CAUTION **The capillary array windows must kept free of any contaminants. Otherwise, high backgrounds and/or drifting baselines will occur. All background counts should be below 6000 RFUs.**

Water used during this procedure must be fresh, distilled, deionized water (18 Mohm/cm water).

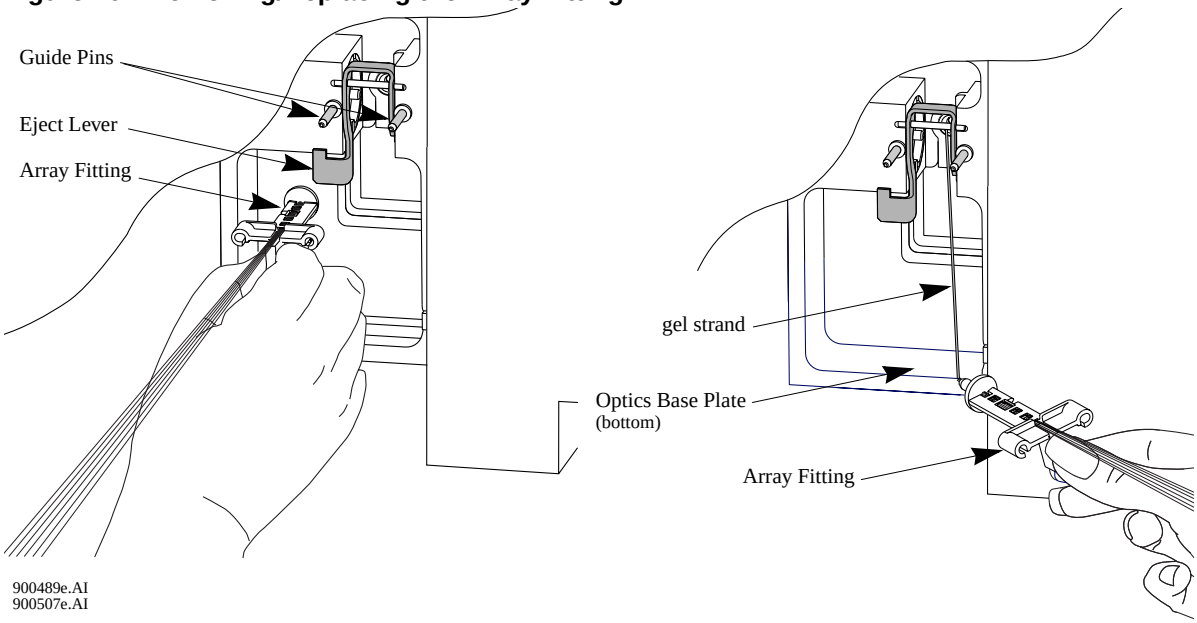
1. Select **Replenish | Release Capillary Array** from the Run menu.
2. Open the Sample Access Cover (Figure 99) and lift to the vertical locking position.
3. Open the Capillary Access Cover and lift to the vertical locking position.
4. Unlatch the two rubber latches holding the Capillary Temperature Control Cover and lift to the vertical locking position.
5. Loosen the Manifold Access Cover captive screw (Figure 100), remove the cover and set it aside.

Figure 100: Manifold Access Cover



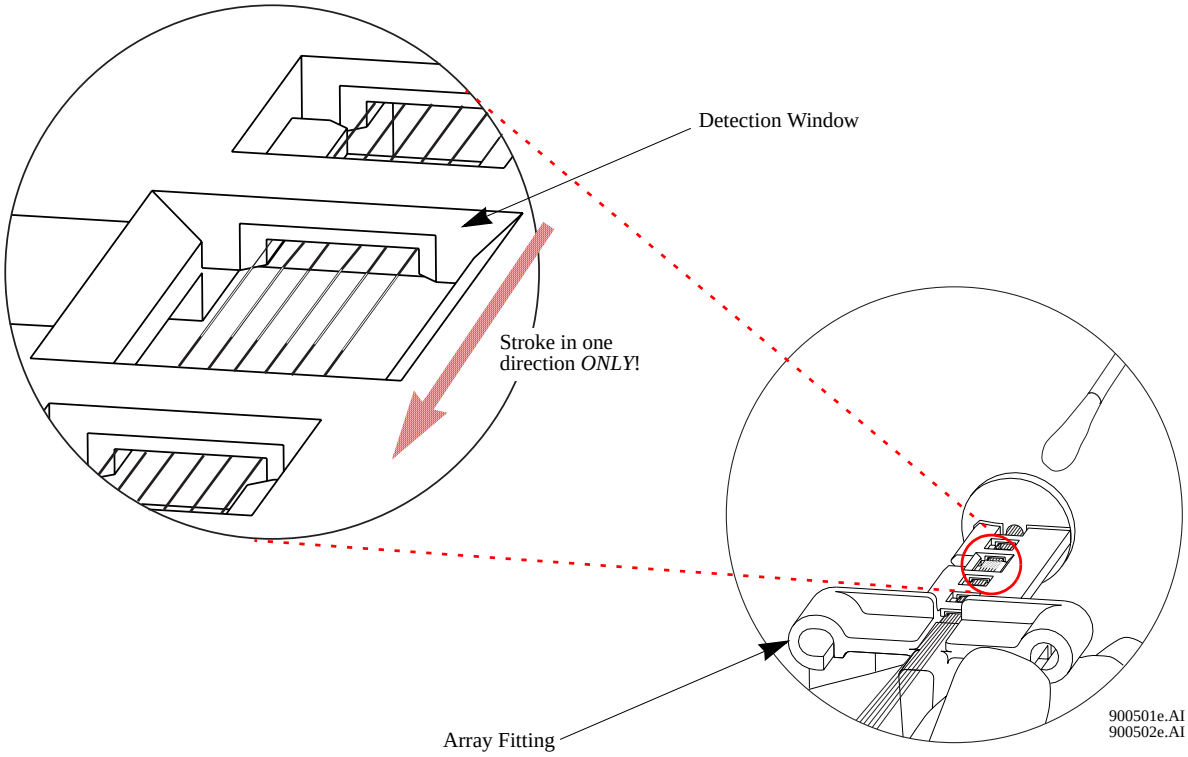
6. Lift the Eject Lever (Figure 101) to release the Array Fitting.
7. Grasp the Array Fitting tab (Figure 101) and then:
   a. Pull the fitting approximately one inch out of the manifold.
   b. Touch the tip of the fitting to the bottom of the Optics Base Plate.
   c. Hold and wait five seconds for the gel strand to dry.
   d. Pull the fitting away from the instrument.
   e. Use a tissue to wipe gel strands off of the instrument.

Figure 101: Removing/Replacing the Array Fitting



8. With the array fitting in hand, blow dust and debris off of the windows with compressed gas (Texwipe Microduster III, P/N: TX2511).
9. Using a water-moistened swab (Texwipe Swab, P/N: TX754B), gently wipe the Detection Window by stroking in one direction *only* as shown in Figure 102.

Figure 102: Cleaning the Detection Window



10. With a new water-moistened swab, repeat wiping on the other side of the window.

11. With a dry swab, gently wipe the windows to remove excess water, again repeating on the backside with a new, dry swab.
12. Blow compressed gas on the windows to remove all excess water.

CAUTION **Care must be taken not to invert the compressed gas bottle, otherwise propellant will contaminate the capillary windows.**

13. If dried gel or other debris remains on the window, repeat this procedure.
14. From the **Remove Capillary** dialog box (Figure 103), select the **Clean Capillaries** radio button and click **OK**.

Figure 103: Remove Capillary Dialog

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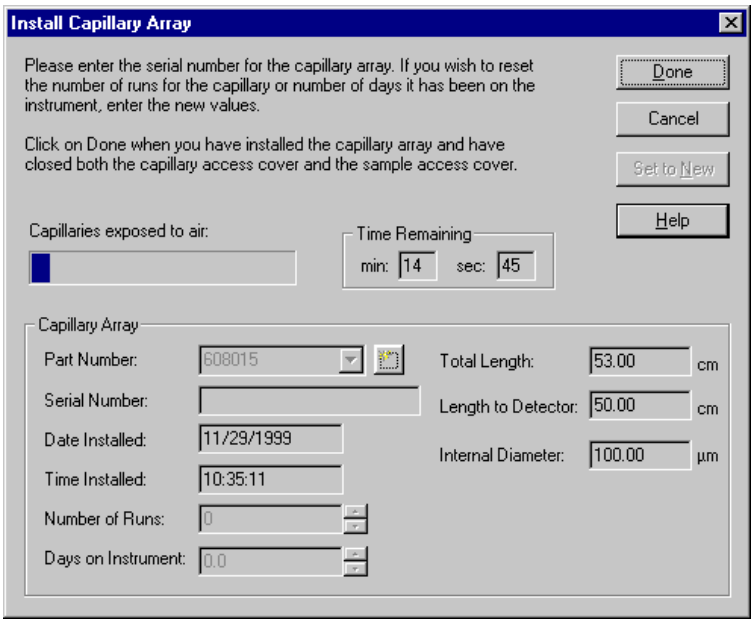
15. While holding the Array Fitting tab (of the clean capillary array), align the Array Fitting with the manifold opening and guide pins. Push the fitting into the manifold until it is completely seated against the bases of the Guide Pins. See Figure 101.
16. Replace the Manifold Access Cover and tighten the captive screw.
17. Lower the Capillary Temperature Control Cover and secure the two rubber latches.
18. Lower the Capillary Access Cover and Sample Access Cover to their locking positions.

Figure 104: Install Capillary Array Dialog



CAUTION **After cleaning the Detection Windows, perform the Optical Alignment procedure and monitor the baseline. If background levels are above 6000 RFU counts, repeat the cleaning process.**

Replacing the Gel Waste Bottle

This procedure assumes that a used (full) waste bottle is being replaced with an empty waste bottle.

1. Remove the cap from the new (empty) waste bottle.
2. Open the Sample Access Cover (Figure 99) and lift to the vertical locking position.
3. With the Gel Waste Bottle 80-90% full, remove the cap and pull the bottle out of the instrument.
4. Place the cap from the new bottle over the full waste bottle and secure.
5. Thread the new bottle onto the cap attached to the instrument and set the bottle into position.
6. Close the Sample Access Cover.
7. Dispose of the full waste bottle according to procedures found in ***"Disposal of the Gel Waste Bottle"*** on page 4-11.

Cleaning the Wetting Tray

1. Rinse the Wetting Tray with deionized water and dispose of the rinse in the liquid waste container.
2. After all wells are clear, allow the empty tray to sit in a ventilation unit for 24 hours.

Filling the Wetting Tray

CAUTION **No more than one 96-well plate should be processed without replenishing the Wetting Tray.**

Periodically check the liquid level in the wetting tray. Liquid level should NEVER be allowed to rise into the eight cannula recesses of the wetting tray lid, nor drop below the fill level indicator line (9 mL minimum). The top surface of the wetting tray lid must remain clean and dry under any and all circumstances.

Replacing the Wetting Tray

Removing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the menu.
2. Open the Sample Access Cover (Figure 99) and lift to the vertical locking position.
3. Remove the Sample Plate and set aside.
4. Rotate the Wetting Tray Retainers outwards to release the Wetting Tray.
5. Lift the Wetting Tray vertically.

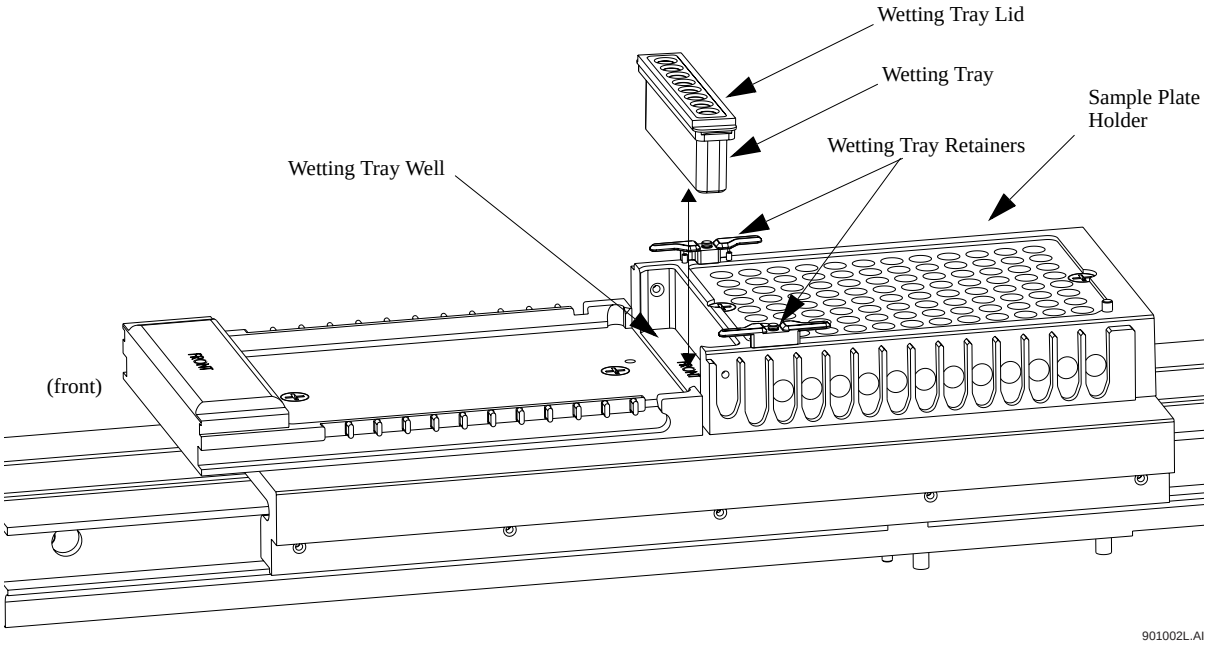
Filling with D.I. Water

1. Remove the lid of the Wetting Station and fill with D.I. water to the indicator line.
2. Close the lid.

Installing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the menu.
2. Open the Sample Access Cover (Figure 99) and lift to the vertical locking position.
3. Insert the Wetting Tray into the receptacle between the Sample and Buffer plates (Figure 107) and then gently press it down into the well.
4. Rotate the Wetting Tray Retainers inwards to lock the Wetting Tray in place.
5. Install the Sample Plate.

Figure 105: Replacing the Wetting Tray

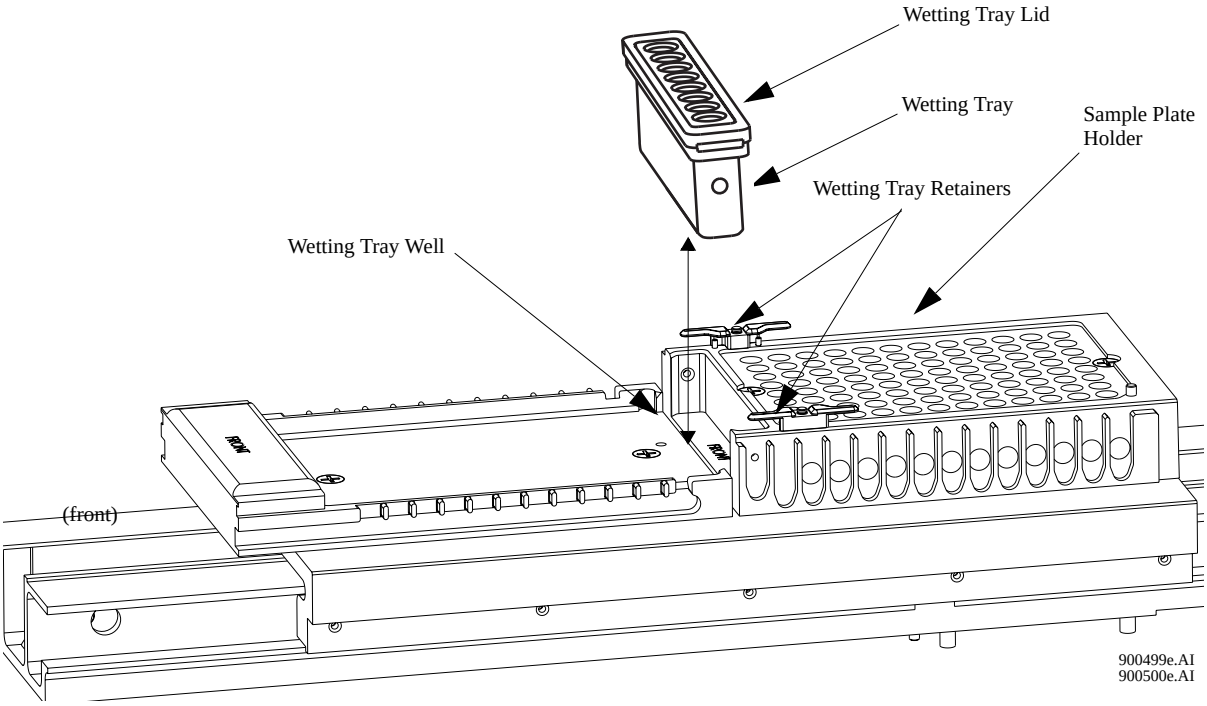


6. Close the Sample Access Cover and then click the **Done** button of the **Replace Wetting Tray** dialog box (Figure 106).

Figure 106: Replace Wetting Tray Dialog

7. Open the Sample Access Cover (Figure 99) and lift to the vertical locking position.
8. Remove the current Wetting Tray from the Sample Plate Holder (Figure 107) and set it aside.
9. Insert the replacement Wetting Tray into the Sample Plate Holder.

Figure 107: Filling the Wetting Tray



10. When finished positioning the tray, close the Sample Access Cover and then click the **Done** button of the **Replace Wetting Tray** dialog box (Figure 108).

Figure 108: Replace Wetting Tray Dialog

Biological Waste Disposal

WARNING **The CEQ 2000 System has been designed to minimize exposure to hazardous chemicals and biological waste. However, care must still be exercised when removing used chemicals and biological samples from the instrument. The information below provides the minimum protocols to use when handling hazardous chemicals and biological waste.**

Disposal of Formamide from the Sample Plate

Multi-Channel Pipettor

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

1. Aspirate 40 mL of formamide from the thermo-cycling plate.
2. Dispense the formamide into a hazardous liquid organic waste container.
3. After all wells are clear, allow the empty plate to sit in the ventilation unit for 24 hours.

WARNING **Dispose of formamide in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Bulk Disposal

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

1. Using a 1L side arm flask as a trap, connect the trap to a vacuum.
2. Attached a pipette to the trap using chemical resistant tubing.
3. Aspirate the formamide from each well and dispose of it in a hazardous liquid organic waste container.
4. After complete removal of all formamide from the plates, allow the empty plate to sit in the ventilation unit for 24 hours.
5. Dispose of the plate in a solid waste container.

WARNING **Dispose of formamide in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Disposal of Buffer/Gel Mixture from the Buffer Plate

Multi-Channel Pipettor

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

1. Aspirate 400 µL of buffer/gel mixture from the 96-well plate.
2. Dispose the buffer/gel mixture into a hazardous liquid organic waste container.
3. Rinse the plate wells with water and dispose of the rinse in the liquid waste container.
4. After all wells are clear, allow the empty plate to sit in the ventilation unit for 24 hours.
5. Dispose of the plate in a solid waste container.

WARNING **Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Bulk Disposal

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

1. Using a 1L side arm flask as a trap, connect the trap to a vacuum.
2. Attached a pipette to the trap using chemical resistant tubing.
3. Aspirate the buffer/gel mixture from each well and dispose of it in a hazardous liquid organic waste container.
4. After complete removal of all buffer/gel mixture from the plates, allow the empty plate to sit in the ventilation unit for 24 hours.
5. Dispose of the plate in a solid waste container.

WARNING **Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Disposal of the Capillary Array

WARNING **After removing the expended capillary array from the instrument, dispose of it in a solid hazardous waste container.**

Disposal of the Gel Cartridge

WARNING **After removing the expended gel cartridge from the instrument, use a lab spatula or wooden dow to push any remaining gel into a liquid hazardous waste container. Dispose of the empty cartridge in a solid hazardous waste container.**

Disposal of Buffer/Gel Mixture from the Wetting Tray

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

1. Pour the buffer/gel mixture into a hazardous liquid organic waste container.
2. Rinse the Wetting Tray with water and dispose of the rinse in the liquid waste container.

WARNING **Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Disposal of the Gel Waste Bottle

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.

Consumable Items List

Table 64 provides a list of the required consumable items for the Sequence Analysis system.

Table 64: Consumable Items Required for Sequence Analysis

Item	P/N	QTY	Description	on Instrument Life	Shelf Life
DTCS Kit (DNA Sequencing RXN Kit)	608000	1	Dye Terminator Cycle Sequencing Kit for 96 reactions. Includes: • DNA polymerase • CEQ Dye Terminators (ddUTP, ddGTP, ddCTP, ddATP) • dNTP Mix Solution • Sequencing Reaction Buffer • pUC18 Control Template • M13-47 Sequencing Primer • Glycogen • Mineral Oil	N/A	1 year @ -20°C (frost free freezer)
CEQ™ Separation Gel I	608010	1	11.5 mL of gel in CEQ 2000 compatible container. Sufficient for 12 runs (96 samples).	2 Days	8 months @ 4°-6°C
CEQ™ Separation Buffer	608012	1	Each container has a screw top and pour tip. The container has enough buffer (30 mL) to fill a CEQ System 96-well, flat bottom Buffer Plate. (Each well being ¾ full.) 4/Pack	fresh, each run	1 year @ 4°-6°C
DNA Separation Capillary Array 33-75B	608087	1	Eight capillaries, 75 µm i.d., 33 cm long, 200 o.d. complete with electrode block and detector array fitting. Ready for installation into CEQ 2000.	4 weeks	8 months @ 4°-6°C

Table 65 provides a list of the required consumable items for the Fragment Analysis system.

Table 65: Consumable Items Required for Fragment Analysis

Item	P/N	QTY	Description	on Instrument Life	Shelf Life
DNA Separation Capillary Array 33-75B	608087	1	Eight capillaries, 75 µm i.d., 33 cm long, 200 o.d. complete with electrode block and detector array fitting. Ready for installation into CEQ 2000.	4 weeks	8 months @ 4°-6°C
CEQ™ Separation Gel I	608010	1	11.5 mL of gel in CEQ 2000 compatible container. Sufficient for 12 runs (96 templates).	2 Days	8 months @ 4°-6°C
CEQ™ Separation Buffer	608012	1	Each container has a screw top and pour tip. The container has enough buffer (30 mL) to fill a CEQ System 96-well, flat bottom Buffer Plate. (Each well being ¾ full.) 4/Pack	fresh, each run	1 year @ 4°-6°C
CEQ™ DNA Size Standard Kit - 400	608098	1	DNA size standard for analysis of fragments up to 400 nucleotides. Includes: • Mineral Oil • DNA fragments of the following sizes labeled with CEQ WellRED fluorescent dye: 60, 70, 80, 90, 100, 120, 140, 160, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, and 420 nucleotides Sufficient for 96 fragment analysis separations.	24 Hours	1 year @ -20°C

Item	P/N	QTY	Description	on Instrument Life	Shelf Life
CEQ™ DNA Size Standard Kit - 600	608095	1	DNA size standard for analysis of fragments up to 600 nucleotides. Includes: • Mineral Oil • DNA fragments of the following sizes labeled with CEQ WellRED fluorescent dye: 60, 70, 80, 90, 100, 120, 140, 160, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, and 640 nucleotides Sufficient for 96 fragment analysis separations.	24 Hours	1 year @ -20°C
CEQ™ Fragment Analysis Test Sample	608105	1	A set of fluorescently labeled DNA fragments of known sizes and defined spacing used to verify the resolution power of the CEQ 2000 fragment analysis system. Sufficient for 24 tests.	24 Hours	1 year @ -20°C
CEQ™ Fragment Analysis Mobility Calibration Standard	608100	1	Four sets of 12 DNA fragments (40-400 nucleotide size range), each labeled with a different CEQ WellRED fluorescent dye. Used for the determination of the effects of the dye labels on fragment mobility. Sufficient for 10 calibrations.	24 Hours	1 year @ -20°C

Materials Required but not Supplied by Beckman Coulter

Sequence Analysis

- Molecular Biology Grade: sterile dH_2O, 95% (v/v) ethanol/dH_2O, 70% (v/v) ethanol/ dH_2O
- 3M Sodium Acetate pH5.2 - Sigma, Cat # 430771
- 100mM Na_2-EDTA pH8.0
- Formamide, UltraPure Bioreagent - J.T. Baker, Cat# 4028-00 or American Bioanalytical, Cat # AB00600
- Mixed Bed Resin AG501-X8(D) - Bio-Rad, Cat# 143-6425
- 0.2µM Nylon filtration unit - Corning Cat, # 430771
- Sterile tubes - 0.5 mL microfuge, 0.2 mL thin wall thermal cycling tubes or plates
- Thermal cycler with heated lid
- Spectrophotometric Grade Methanol
- Texwipe Swabs, P/N: TX754B (VWR)
- Texwipe Microduster III, P/N: TX2511B (VWR)

Fragment Analysis

- Thermal cycling device w/ heated lid and refrigerated microfuge
- Molecular Biology Grade: sterile dH_2O, 95% (v/v) ethanol/dH_2O and 70% (v/v) ethanol/dH_2O
- Formamide, UltraPure Bioreagent - J.T. Baker cat. # 4028-00
- Mixed Bed Resin AG501-X8(D) - Bio-Rad cat. # 143-6425
- 0.2 µM Nylon filtration unit - Corning cat. # 430771
- 20 mg/mL Glycogen - Boehringer Mannheim cat. # 901 393
- 3M Sodium Acetate pH 5.2 - Sigma cat. # S7899
- Sterile tubes - 0.5mL microfuge, 0.2mL thin wall thermocycling tubes
- PCR enzyme and buffer
- Labeled primers (available from Research Genetics)
- Thermal cycling plates and caps
- Pipette Tips with barrier

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